INNOVATIVE STUDIES IN AGRICULTURE AND NATURAL SCIENCES

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PREFACE

Dear Scientists,

Agriculture and natural sciences have always played a fundamental role in sustaining human life, ensuring food security, and understanding and protecting the natural environment. Today, rapid population growth, climate change, declining natural resources, environmental pressures, and increasing demands for sustainable production have made scientific research in these fields more important than ever. Addressing these complex challenges requires not only advances within individual disciplines but also innovative approaches that integrate knowledge, methods, and technologies from different fields of science. Recent developments in science and technology have opened new perspectives in many areas, ranging from agricultural production and biotechnology to genetics, molecular biology, environmental sciences, food systems, and the sustainable management of natural resources. These developments provide valuable opportunities to improve productivity and quality, enhance resilience to changing environmental conditions, and develop more sustainable approaches to the use and conservation of biological and natural resources.

Innovative Studies in Agriculture and Natural Sciences has been prepared to bring together current research, scientific perspectives, and innovative approaches from different fields of agriculture and natural sciences. Comprising 22 chapters, this volume addresses a broad range of topics and presents recent scientific developments, methodologies, applications, and research findings from different perspectives. Rather than focusing solely on existing knowledge and current challenges, the book also aims to contribute to the development of new perspectives and research directions. By bringing together studies from different areas of expertise, we hope that this volume will encourage further scientific discussion, interdisciplinary collaboration, and innovative research in agriculture and natural sciences.

As editors, we sincerely thank all contributing authors for sharing their knowledge, experience, and scientific work. Their valuable contributions have made it possible to present a diverse and comprehensive collection of studies to the scientific community. We hope that ***Innovative Studies in Agriculture and Natural Sciences*** will serve as a useful reference for researchers, academics, students, and all readers interested in current and emerging

developments in agriculture and natural sciences, and that it will contribute to future studies in these rapidly evolving fields.

Sincerely,

September, 2026

Prof. Dr. Ahmet KAZANKAYA

Assoc. Prof. Dr. Mevlüde Alev ATEŞ

CHAPTER 1

THE INVASION OF AQUATIC ECOSYSTEMS BY PLASTIC WASTE

Prof. Dr. Fazıl ŞEN[1]

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1. INTRODUCTION

Due to the large quantities of plastic waste found in highly populated and industrialized regions, some scientists have referred to the period in which we live as the Plastic Age (Akhan & ark., 2022).

Due to the numerous advantages, they offer, plastics are produced in large quantities worldwide and are used in almost every field. Historically, people initially used containers made of clay and wood; however, plastics have replaced these materials today because they are easier and less expensive to produce and transport. As plastics are now used in almost every area of daily life, the idea of a world without plastics has become almost impossible to imagine. The convenience provided by plastics has made people increasingly dependent on them. Plastic waste has been discarded in such large quantities and across such vast areas that it can even be found within glaciers and in the bottom sediments of the Mariana Trench, the deepest point on Earth (11,035 m) (Yurtsever, 2018). This pollution affects marine tourism, fisheries, the food industry, humans, and even infants through breast milk.

Among the most prevalent forms of plastic pollution, microplastics, which vary in size and shape in the environment, have become a major threat to living organisms and the environment due to their invisibility to the naked eye, ubiquitous presence, and durability. Consequently, microplastics have become one of the most extensively studied topics in this field. Plastic pollution, which is entirely anthropogenic in origin, has become a widespread problem encountered in virtually all environments, particularly aquatic ecosystems. As plastics break down into very small particles, they can be readily dispersed over large areas and enter water resources, where they are ingested by living organisms and can adsorb harmful substances present in the water, thereby facilitating their transfer through the food chain. For these reasons, combating plastic waste through a holistic approach involving all stakeholders has become increasingly important (Yurtsever, 2018).

It is known that plastic waste released into the environment is transported to the surface and depths of the oceans, forming massive plastic waste islands in some oceanic regions (Fig. 1). It is even estimated that, if this trend continues, geological rocks may eventually contain plastic layers due to the accumulation of plastic waste (Yurtsever, 2018; Ocak, 2022).

Figure 1. Plastic waste accumulated in the oceans (UNEP, 2021; Ocak, 2022)

2. WHAT IS PLASTIC?

Plastic is a term used to describe a variety of synthetic or semi-synthetic materials. The term "plastic" is derived from the Greek-Latin words *plastikos*, *plastos*, and *plassien*, meaning capable of taking a desired shape and being molded or kneaded (Freinkel, 2011; Songür-Dağ, 2015; Hedeyati et al., 2022; Ciner et al., 2023). Plastics are materials composed of high-molecular-weight organic polymers. Like all polymers, their basic structure consists primarily of carbon atoms. They are produced through polymerization processes from natural organic materials, particularly petroleum, as well as coal and natural gas. They can also be produced synthetically or from cotton residues. Due to their moldability and plasticity, plastics can be transformed, pressed, or shaped into films, fibers, sheets, tubes, bottles, containers, and many other products. Owing to their durability and resistance to degradation, they persist in the environment for long periods. Petroleum-based plastics are now widely used in both personal and industrial activities (Güler & Çobanoğlu, 1997; Kılıç & Yüce, 2014; WHO, 2019; Kayan & Küçük 2020; UNEP, 2021; Akhan et al., 2022; Alarif et al., 2023; Önder, 2023; UNEP, 2023; Marcharla et al., 2024; Çelebi & Gökmen 2025; Karak et al., 2025; Jachimowicz et al., 2026).

Due to their flexibility, light weight, low cost, ease of processing, electrical insulating properties, resistance to many chemicals, and the ease and low cost of their production and transportation, as well as their convenience in use, plastics are widely used in food and beverage packaging, shopping bags, and various products and materials. Owing to these properties, plastics are now widely used in almost every aspect of daily life (Yurtsever, 2018; Kayan & Küçük, 2020; Ciner et al., 2023)

Approximately 40% of plastics are used in the packaging industry, and

single-use plastics in particular, such as bags, straws, packaging materials, PET bottles, and cups, are rapidly exacerbating the plastic waste problem. Some specially engineered durable plastics are highly resistant to environmental factors and ultraviolet (UV) radiation (Jansen et al., 2024).

3. HISTORY OF PLASTIC PRODUCTION

The earliest uses of rubber, considered the precursor to plastics, can be traced back to the ancient Mesoamerican civilizations, including the Maya, Aztecs, and Incas (2000 BC-900 AD). These civilizations collected natural rubber and used it to make figurines and sculptures. Until the nineteenth century, when the development of modern thermoplastics began, natural polymers were used in the form of horn, waxes, natural rubber, and resins (Andrady & Neal, 2009; Önder, 2023).

In the United States, during the nineteenth century, ivory was used to manufacture billiard balls, and the difficulty of obtaining ivory led to research into alternative materials. As a result of these efforts, John W. Hyatt adapted plastic for use in billiard balls in 1869 and patented it in combination with celluloid. Celluloid plastic was later adopted in the film and photography industries. Another important application of plastics has been in the music industry. In the nineteenth century, Edison invented the phonograph cylinder, enabling sound to be recorded and played back using wax. Later, records, cassette tapes, and CDs were developed. The use of plastic celluloid as a replacement for paper reels also contributed to the development of the film industry (Kayan & Küçük, 2020; Neves et al., 2023).

Today, plastics are used in almost every aspect of life, from healthcare and the kitchen to industry and agriculture. The production of Bakelite, the first synthetic plastic developed by Leo Hendrik Baekeland in New York in 1907, marked the beginning of synthetic plastic materials. Bakelite is a material that can be easily molded, retains its shape after cooling, and is non-biodegradable. Due to its electrical insulating properties, it has been used in lighting equipment, plugs, and electrical sockets. Bakelite quickly became a standard material across various industrial applications and, from the 1930s and 1940s onward, was also used in the production of radios and phonograph records (ACS, 1993; Kayan & Küçük 2020).

In 1922, H. Staudinger developed polymers by linking plastic molecules together, introducing polymerization into the chemical industry. During the 1930s and 1940s, polyethylene (PE) was discovered as a material that was insoluble at room temperature and resistant to gas transmission, and it played an important role in the wartime industry. The Second World War had a major influence on the widespread use of plastics today. During this period, PE was extensively used for insulating electrical wiring in aircraft radar systems, nylon for parachute production, acrylic for aircraft windows, and plastics for helmet production in the military industry. Following the end of the war, plastic manufacturing companies began expanding into different fields, including the production of products such as Barbie dolls. In the 1990s, the widespread adoption of plastic products in the healthcare sector as alternatives to glass products that were difficult to sterilize and easily breakable, such as intravenous solution bottles, syringes, and medicine tubes, became another factor contributing to the increased use of plastics (Güler & Çobanoğlu, 1997; Tullo 2013; Kayan & Küçük, 2020).

The German company BASF initiated the commercial production of polystyrene (PS) in the 1930s. In the United States, Dow Chemical Company began using PS in 1954 as an excellent insulating material for buildings and as a moldable packaging material. While PS is widely used in consumer goods, it is also used in industrial packaging to protect electronic products, such as televisions, washing machines, and lighting equipment, as well as valuable items during transportation (Echte et al., 1981; Güler & Çobanoğlu, 1997; Kayan & Küçük, 2020).

Polyethylene (PE), which is widely used today, was first synthesized in 1935 as a low-density resin. PE production processes have become more sophisticated and cost-effective over time (Güler & Çobanoğlu, 1997; Andrady & Neal, 2009; Önder, 2023).

The first plastic sandwich packaging began to be used in 1957, while single-use plastic products such as plates, forks, spoons, and cups were introduced in the 1960s. PET bottles were initially used for carbonated beverages, and the first patent for PET bottles was granted in 1973. Today, PET bottles have become an important part of everyday life (Güler & Çobanoğlu, 1997; Kayan & Küçük, 2020; Kumari et al., 2024).

During the first 50 years of the twentieth century, modern plastics expanded considerably, with at least 15 new classes of polymers being synthesized. Annual plastic production increased from 1.5 million tons in the 1950s to 450 million tons in 2020, with only a small proportion being recycled (OECD, 2022a). Global annual per capita plastic consumption increased from 2 kg in 1974 to 43 kg today. Between 1950 and 2015, 8.3 billion tons of plastic were produced, of which approximately 6.3 billion tons became waste. According to a report, plastic production in Türkiye reached 9.46 million tons. Global plastic consumption is projected to reach 33 billion tons by 2050, with an estimated 12 billion tons becoming waste (Kayan & Küçük, 2020; Bozdaş et al., 2020; OECD, 2022b).

Of all the plastics produced to date, only 9% has been recycled, 12% has been incinerated, and 79% has accumulated in landfills or the natural environment. It is estimated that 8–12 million tons of plastic waste enter the oceans each year. As of 2020, the global mass of produced plastics had exceeded the total biomass of all terrestrial and marine animals. Some researchers have suggested that, by 2050, there could be more plastic than fish by weight in the oceans (Jambeck et al., 2015; Geyer et al., 2017; OECD, 2022b).

Multinational companies are contributing to the widespread use of plastics by offering many products, including ready-to-eat foods, beverages, cosmetics, and cleaning products, in single-use packaging. A striking example is Coca-Cola, which owns more than 500 brands and sells more than 110 billion single-use bottles annually, resulting in more than 3,400 bottles being discarded into the environment every second. The company generates annual profits of more than £6.5 billion (Greenpeace, 2017; Kayan & Küçük, 2020).

4. TYPES, CLASSIFICATION AND PROPERTIES OF PLASTICS

Plastics can be classified into two groups, semi-synthetic and fully synthetic, according to the type of polymer from which they are derived. Semi-synthetic plastics are those whose basic chain structure is derived from a natural product such as cellulose. Fully synthetic plastics constitute a group in which the basic chain structures are chemically formed from monomers or small molecular units. Polymerization is the process by which polymers are formed

from monomers, their constituent building blocks. In addition, the low cost, transparency, and oxygen and moisture barrier properties of plastics make them excellent packaging materials (Güler & Çobanoğlu, 1997; Yurtsever, 2018).

Plastics can be classified into two categories according to their recyclability: thermoplastics and thermosets. Thermoplastics can be heated and subsequently reprocessed to produce new products. Thermoset plastics can be shaped after being melted; however, once they have been formed, they cannot be melted and reshaped again (Ciner et al., 2023).

The resin identification and recycling codes, numbered from 1 to 7, used for the chemical identification of plastics, along with their names and areas of application, are presented in Table 1.

Table 1. Names, resin identification and recycling codes, and applications of the most commonly used plastics (Yurtsever, 2018)

Code	Plastic Name	Applications
1	PET, PETE (Polyethylene terephthalate) 1.37-1.45 g/cm³	Widely used. Water bottles, soft drink bottles
2	PE-HD, HDPE (High-density polyethylene) 0.94-0.96 g/cm³	Widely used. Cleaning product, laundry detergent and shampoo bottles, milk bottles
3	PVC (Polyvinyl chloride) 1.16–1.58 g/cm³	Manufacturing of pipes, profiles, etc.; plastic films, bottles, cups
4	PE-LD, LDPE (Low-density polyethylene) 0.91-0.93 g/cm³	Widely used. Plastic bags, bottles
5	PP (Polypropylene) 0.83-0.90 g/cm³	Automotive components and garden furniture
6	PS (Polystyrene) 0.96-1.05 g/cm³	Packaging, electronic equipment and household appliances, insulation
7	Other-Various plastics or mixtures thereof (e.g., polycarbonate, acrylic)	These products may contain BPA. Caps, medical storage containers, water carboys, cups, forks, kitchen utensils, automotive applications, etc.
81	Composite (Paper and cardboard + plastic)	Packaging
82	Composite (Paper and fiberboard + plastic + aluminum)	Packaging
84	Composite (Paper and cardboard + plastic)	Packaging (food products)
85	Composite (Paper and fiberboard + plastic + aluminum + tin)	Packaging
90	Composite (LDPE plastic + aluminum)	Packaging (food products)

Among the most commonly used types of plastics are polyethylene (PE), polypropylene (PP), polyethylene terephthalate (PET), polystyrene (PS), and polyvinyl chloride (PVC). In addition, there are numerous plastic polymers with different types and properties, such as polyester (PES), ABS (acrylonitrile butadiene styrene), PMMA (polymethyl methacrylate, acrylic, Plexiglas), ASA (acrylonitrile styrene acrylate), PC (polycarbonate), PUR (polyurethane), POM

(polyoxymethylene, acetal), and SAN (styrene acrylonitrile), among others. Plastics are also used in the manufacture of composite packaging materials (Table 1). Plastics, when combined with materials such as paper, cardboard, aluminum, and tin, can increase the durability of composites. However, this makes recycling more difficult. If the code "**C/**" is present on the packaging, it indicates that the material is made of a composite and is disposed of with non-recyclable materials. For example, composite materials such as C/PAP (84) and C/LDPE (90) are among the composites used in food and beverage packaging (Yurtsever, 2018).

Plastic waste can be classified into five groups according to size: nanoplastics (1 µm–0.5 mm), microplastics (0.5–5 mm), mesoplastics (5–20 mm), macroplastics (20–100 mm), and megaplastics (>100 mm). According to their shape, they are classified as fibers, fragments, filaments, pellets, films, and foams. They may occur in various geometric forms, including round, spherical, oval, rectangular, cylindrical, disk-shaped, and amorphous forms. According to their type, they are referred to as granules, pellets, particles, filaments-fibers, films, long or short fragments, flattened particles, flakes, fractured particles, and Styrofoam (foam).

The degree of color fading or brightness of a microplastic, as well as the roughness or glossiness of its surface, i.e., its degree of abrasion, can provide information about when it entered the aquatic environment and whether it has undergone degradation (aging and deterioration) (Thomson, 2015; Kayan & Küçük, 2020; Akhan et al., 2022).

The major properties of microplastics include hydrophobicity, buoyancy, the ability to transport pollutants, adsorption, UV-induced photo-oxidative degradation, thermo-oxidative degradation, biological or thermal fragmentation, and durability. Durability is an environmental disadvantage. Their degradation is largely time-dependent, and their effects do not diminish as they fragment into smaller particles. They have long persistence times (Table 2). Since they are less dense than water, they can float on the water surface and travel long distances. Under the influence of UV radiation, degradation and hydrolysis cause plastics to lose their elasticity, allowing them to be transported by wind and waves and gradually fragmented into smaller particles (Hidalgo-Ruz et al., 2012; Wright et al., 2013; Yurtsever, 2018; Chamas et al., 2020).

Table 2. Estimated persistence times of plastics in the environment

Type of Plastic	Estimated Persistence Time in the Environment
Plastic bag	20–300 years
PET bottle	400–500 years
Fishing net	600+ years

General properties of plastics and reasons for their use: 1) They are lightweight and float in water; 2) they are lipophilic (soluble in fats); 3) they are polymers species; 4) they are resistant to many chemicals; 5) they are easy to process and shape; 6) they are resistant to heat and cold and are insulators against electric current; 7) some plastics can be reused and regenerated; 8) they can adsorb and transport chemicals; and 9) their properties can be improved by using additives (bisphenol A, phthalates, Pb and Cd metals, fungicides, etc.), allowing highly durable plastics to be produced (Yurtsever, 2018).

4.1. Microplastics

Waste plastics break down into particles of microscopic size under various environmental influences. The term microplastic was first used in 1968 in a report by the Air Force Materials Laboratory to describe particles formed as a result of the deformation of plastic materials under high stress (Ingram et al., 1968). The presence of microplastics in aquatic environments was first reported when numerous small floating plastic particles were found in the surface waters of the Sargasso Sea (Carpenter & Smith, 1972; MAG, 2026).

The term microplastic has been defined differently by various researchers. Thompson et al. (2004) were the first to define microplastics as plastic particles smaller than 5 mm, while other studies have reported that their size ranges from 1 to 20 mm. In their study, Browne et al. (2011) reported that microplastics accumulate in the marine environment and are smaller than 1 mm. In recent years, microplastics have been defined as any synthetic solid particle or polymeric matrix ranging in size 1 μm-5 mm, insoluble in water, originating from either primary or secondary sources, and potentially having regular or irregular shapes (Thompson, 2015; Yurtsever, 2018; Frias & Nash, 2019).

4.1.1. Types of Microplastics

Microplastics in aquatic environments consist of those formed through the fragmentation of larger plastic items and those industrially manufactured

for various purposes. To distinguish between these two types, industrially produced microplastics are referred to as **primary microplastics**, whereas those resulting from the fragmentation of larger plastics are called **secondary microplastics** (Cole et al., 2011; Yurtsever 2018; MAG, 2026).

Primary microplastics are produced at microscopic sizes and are generally intentionally manufactured by the plastics industry for use in cosmetics, personal care products such as makeup, body and facial cleansers, dermal exfoliants (skin cleansers), cleaning agents, and abrasive blasting machines. They also include small, round microbeads, plastic pellets produced for industrial applications, synthetic fibers used in handicrafts and textile materials, and glitter. In addition, microplastics are added to industrial cleaning products to remove rust or paint (Fig. 2).

Secondary microplastics are plastics that are weathered, fragmented, and broken down into smaller particles as a result of environmental factors such as UV radiation, wind, and aquatic environments. They consist of irregularly shaped plastic fragments formed through the degradation of larger plastic items, such as plastic bags, crates, bottles, and, particularly, ropes and fishing nets (Fig. 3).

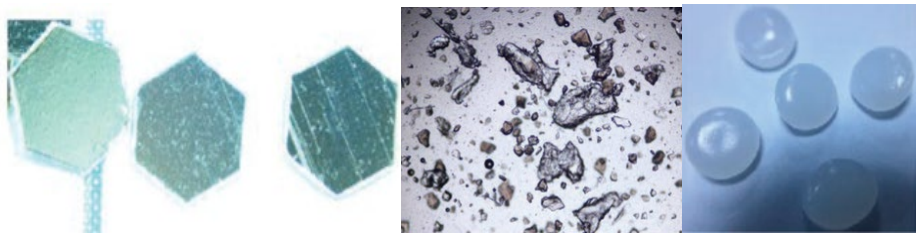

Figure 2. Primary Microplastics (M. Yurtsever, 2018)

Figure 3. Secondary microplastic sources (M. Yurtsever, 2018)

4.1.2. Formation of Microplastic

Microplastics are one of the most critical dimensions of plastic pollution. Over time, the structural integrity of plastics gradually deteriorates under various physical, chemical, and biological effects, as well as mechanical forces such as UV radiation and tides, causing them to break down into smaller particles. High-density polyethylene (HDPE), low-density polyethylene (LDPE), polypropylene (PP), and nylon primarily undergo degradation through UV photo-oxidation and thermo-oxidation, after which the resulting products can further break down into micro- or nanoscale particles. Although the cold temperatures of the marine environment may inhibit photo-oxidation, plastics can become brittle over time because they are exposed to high levels of oxygen and direct sunlight on beaches. In one study, a 1 cm^2 polystyrene coffee cup lid was exposed to ultraviolet radiation at 320-400 nm for 24 hours per day at 30 °C for 56 days. The study demonstrated that the lid could produce

approximately 126 million nanoparticles per milliliter, with an average size of 224 nm. Based on these findings, it can be suggested that these small plastic particles can readily disperse throughout the entire water column and may be ingested by many marine organisms after being mistaken for food. Considering the enormous use of plastic materials in our daily lives, the potential harm caused by microplastics as particles, as well as the harmful effects associated with their chemical components, becomes increasingly evident (Cole et al., 2011; Lambert & Wagner, 2016; Chamas et al., 2020).

5. PLASTIC FOOTPRINT AND POLLUTION

Footprint is a concept that symbolizes the pressure exerted by humans on the productive natural areas and nature that they use. The total amount of plastic used by an individual (plastic bottles, computer components, microbeads, bags, nylon clothing, etc.) is defined as the **Plastic Footprint** (Ciner et al., 2023). Plastic pollution has been found to reach many places, from uninhabited islands and polar regions to the Mariana Trench, the deepest point on Earth (11,035 m). For example, microplastics have been detected in deep-sea sediments in the Western Pacific at depths of 4,601-5,732 m (240 particles per kg dry weight) (Huang et al., 2021) and in coral reefs around the Xisha Islands in the South China Sea (0.2–45.2 particles/L) (Wei et al., 2025). Microplastics/nanoplastics have also been detected in snow in both the northern and southern polar regions (Yurtsever, 2018 and 2019).

Plastic pollution is the physical, chemical, and biological impact of synthetic polymer-based materials on the Earth and ecosystems, adversely affecting wildlife, the environment, and humans as a result of their production, consumption, and waste management processes. The widespread use of plastics, together with inadequate waste management, has resulted in persistent plastic pollution. It can be stated that approximately 6.3 billion tons of plastic waste were generated between 1950 and 2015, of which only 9% was recycled, 12% was incinerated, and the remaining 80% was accumulated in landfills or released into the natural environment. An additional 5–12 million tons of plastic pollution enter ocean basins every year. It is estimated that 330,000 tons of plastic are released annually from China's Yangtze River into the Pacific Ocean, while 115,000 tons of plastic are released from India's Ganges River into the Indian Ocean. The majority of plastic waste accumulating in marine

and terrestrial environments consists of single-use plastics such as plastic bottles, bottle caps, cigarette butts, plastic bags, and straws. More than 450 million tons of plastic are produced each year, and a large proportion of it is released into the natural environment (Jambeck et al., 2017; Geyer et al., 2017; Yurtsever, 2018; Huang et al., 2021).

The main sources of plastic pollution include single-use plastic packaging, plastic bags, plastic bottles, plastic containers and plates, synthetic textile products, fishing equipment, etc. (Fig. 4, 5 and 6).

Figure 4. Images of plastic pollution in Ghana, Madagascar, and Vietnam (wikipedia.org, UNEP, 2021)

Figure 5. Aquatic organisms entangled in ghost nets (bianet.org, 2026)

Figure 6. Plastics and human. Photographs By Randy Olson, (Parker, 2026)

5.1. Causes of Plastic Pollution

One of the main causes of plastic pollution is the widespread use of *single-use plastic products* (bags, straws, cups, packaging materials) in all areas of life due to their light weight, durability, low cost, and other advantages.

Approximately 400 billion plastic bags are used annually in the United States. These bags are discarded after short-term use and are often not recycled. *Low recycling rates*, *inadequate waste management systems*, and *unconscious consumption habits* contribute to increasing plastic pollution. In particular, the *fast-consumption culture* that has emerged with the acceleration of modern life encourages the use of more packaged products. In addition, *inadequate waste management*, *excessive plastic production*, *plastic waste trade*, *fishing gear*, *leakage from plastic manufacturing facilities*, and *illegal dumping activities* can be listed among the major causes. Today, in developed countries, there is an average of 100 kg of plastic per person. Most plastic waste accumulates in seas and oceans, and according to one estimate, by 2050 there may be more plastic waste than fish in some seas. The increase since the 1970s has reached such a level that plastics currently account for 11% of the world's waste. According to a report, plastics constitute 95% of the waste in the Mediterranean. Plastic fibers entering wastewater systems from both textile production and the washing of these products, as well as microbeads used in the production of detergents, cosmetics, personal care products, etc., are major causes of pollution in aquatic environments. Microbeads have been banned in many countries. Cosmetics containing microbeads include widely consumed products such as facial cleansing gels, peeling gels, shower gels, shampoos, soaps, toothpastes, eyeliners, mascaras, lip glosses, deodorants, and sunscreens. In 2017, microbeads added to cosmetics were banned in the United States, and in 2018 in the United Kingdom, Canada, Australia, and almost all other countries. In December 2018, the European Union banned single-use plastics, including cotton buds, straws, cutlery, plates, cups, beverage stirrers, balloon sticks, oxo-degradable plastics, and commonly used polystyrene containers, plates, and cups (Eriksen et al., 2014; Jambeck et al., 2015; Chiba et al., 2018; Yurtsever, 2018; Gündoğdu & Çevik, 2019a; Gündoğdu & Çevik, 2019b; Bozdaş et al., 2020; Kayan & Küçük, 2020; OECD, 2022b; Ciner et al., 2023; SıfırAtık, 2026; AKD, 2026).

5.2. Plastic Bag Use and Pollution

One of the most common forms of plastic use is plastic bags. The first use of plastic bags dates back to 1982. However, the idea and patent for using plastic as packaging belonged to the Swedish scientist S. G. Thulin in 1965.

The first supermarket chain to use plastic bags was the U.S.-based Safeway. According to the United Nations, 1–5 trillion plastic bags are used annually. Other studies have reported the annual number of plastic bags used per person as 440 in Türkiye, 375 in the United States, 299 in Australia, and 154 in New Zealand. These figures represent significant sources of environmental pollution. Plastic bags discarded into the environment can block waterways, causing flooding, and can reduce the recreational value of coastal areas by creating an unsightly appearance. This situation has led to measures being taken against the use of plastic bags (Jambeck et al., 2015; Songür-Dağ, 2015; UNEP, 2021; Muposhi et al., 2022).

Due to the environmental damage, they cause, more than 60 countries have recently initiated efforts to reduce the use of plastic bags and have introduced various bans or taxes (ÇöpüneSahipÇıkVakfı, 2019; Muposhi et al., 2022). To give a few examples from around the world, plastic bags were first banned in the Indian state of Sikkim in 1998, followed by Taiwan and Bangladesh in 2002, San Francisco in the United States and Modbury in the United Kingdom in 2007. These were followed by China in 2008, Italy and Malaysia in 2011, Cameroon in 2014, and Germany and EU countries in 2016. While European countries generally impose charges on plastic bags, Asian and African countries have generally introduced bans. In Türkiye, a charge was introduced for plastic bags as of January 1, 2019, and plastic bags have since been provided to consumers for a fee in supermarkets and other stores. As of the end of 2025, this charge was increased from 25 Kuruş to 1 Turkish lira per bag (ÇöpüneSahipÇıkVakfı, 2019; Nielsen et al., 2019; UNEP, 2021; Muposhi et al., 2022; Ministry of Trade, 2026). Based on my personal observations, the introduction of a charge does not encourage people to take any measures regarding their use of plastic bags.

Plastic bags are provided for a fee or through taxation in countries including Germany, Belgium, Bulgaria, Denmark, Sweden, Finland, the United Kingdom, France, Greece, Hungary, Poland, Malta, Italy, Portugal, Estonia, Ireland, Luxembourg, the Netherlands, Slovakia, Slovenia, Scotland, the Czech Republic, Romania, Serbia, Türkiye, and other European countries, as well as the United States, Canada, Japan, Malaysia, and Botswana. Countries that have imposed total or partial bans include Pakistan, Albania, Georgia, Australia, South Korea, Cameroon, Singapore, India, Taiwan, Benin, Burkina Faso, the

Republic of the Congo, Gabon, Gambia, Madagascar, Senegal, Togo, and Bhutan (ÇöpüneSahipÇıkVakfı, 2019). However, in some countries such as India and Bangladesh, although bans have been introduced through legislation, this situation is not consistent with actual implementation.

5.3. Ways of Plastic Contamination in Aquatic Environments

Plastics are transported into aquatic environments through air, wind, and rivers. Within the water, they are dispersed by currents, waves, and aquatic organisms. They are transported to freshwater systems through discharges from wastewater treatment plants, sewage systems, and rainwater and irrigation water from agricultural areas, and from there they can reach seas and oceans (Fig. 7). It is estimated that approximately 80% of plastic waste in the oceans originates from land-based sources, while around 18% originates from fishing and coastal tourism activities (Wayman & Niemann, 2022; Akhan et al., 2022).

As land-based sources, fibers from materials used in clothing can enter water during washing, while detergents containing plastic-based particles used in washing can also introduce plastic waste into aquatic environments. In one study, wastewater from washing machines was examined, and it was determined that a single garment could release approximately 1,900 microplastic particles in one wash. In another study conducted on facial scrubs, it was found that between 4,500 and 94,500 microbeads could be released during a single use (Browne et al., 2011; Ocak, 2022). Other examples of land-based sources include road-marking paints, tire-wear particles, artificial turf, and agricultural waste. The direct discharge of land-based waste into aquatic environments also constitutes an important land-based source. Domestic and industrial wastewater contains large amounts of microplastics. Although treated wastewater is also considered one of the pathways through which plastics enter aquatic environments, well-equipped treatment plants can effectively retain most microplastics. If plastic materials are used in drinking water treatment plants and distribution networks, they may undergo corrosion over time and become a source of microplastics (WHO, 2019).

Figure 7. Pathways of plastic waste entering waters (utilizing artificial intelligence tools)

6. THE EFFECTS OF PLASTIC POLLUTION ON FRESHWATER AND MARINE ECOSYSTEMS

The presence of plastic waste in the oceans began to be reported in the 1970s. In subsequent years, as scientific studies increased, their ecological consequences also began to emerge, and it was revealed that they affect organisms living in aquatic environments, including crustaceans, arthropods, mollusks, marine and freshwater fish, aquatic mammals, waterbirds, turtles, and other organisms. It has been determined that more than 2,000 aquatic species are exposed to lethal effects due to plastic ingestion or entanglement (Fig. 8-9) (Carpenter & Smith, 1972; Andrady & Neal, 2009; Yurtsever, 2018; Akhan et al., 2022)

Plastics derived from fossil-based materials such as petroleum and coal undergo fragmentation in marine environments through mechanical and chemical corrosion, causing serious problems. It has been reported that 4% of crude oil is currently used for plastic production and that toxic gases are released into the environment during production. At the same time, while plastic waste causes serious problems in the digestive systems, it can also lead to injuries and deaths through entanglement in ghost nets (Fig. 8-9), physically damage coral reefs, and disrupt ecosystem balance (Durak, 2016; Kayan & Küçük, 2020)

Figure 8. Ghost nets and sea turtles in the oceans (Theoceancleanup, 2026)

Figure 9. A seahorse off the coast of Sumbawa Island in Indonesia (Ocak, 2022)

Plastics entering ocean waters through eddies caused by high-pressure systems are transported by currents toward the center of the eddies, where they accumulate and effectively form garbage patches. The best-known and most extensively studied garbage patch in the oceans is the Great Pacific Garbage Patch. This ocean garbage patch begins approximately 1,600 km offshore from the Pacific coast of North America and extends hundreds of kilometers westward, covering an area of 1.6 million km². The major plastic garbage patches formed in the oceans are shown in Fig. 10 (Theoceancleanup, 2022).

Figure 10. Plastic islands in the oceans (Theoceancleanup, 2026)

Another dimension of plastic pollution is its visual impacts. Plastic waste washed ashore negatively affects both natural beauty and tourism. The fisheries sector is also adversely affected by plastic waste. Waste entangled in fishing nets increases costs and contributes to the decline in fish stocks. Plastic waste becomes integrated into marine food webs and is transported across trophic levels. Some studies have shed light on the size- and shape-dependent accumulation and toxicity of micro/nanoplastics in zebrafish (UNEP, 2021; Önder, 2023; Gençağ, 2025; Yön-Ertuğ et al., 2025).

Microplastics can adsorb toxic organic chemicals such as organochlorine pesticides, persistent organic pollutants, endocrine-disrupting chemicals, and antibiotics, as well as heavy metals, thereby presenting aquatic organisms with a complex mixture of contaminants. Furthermore, due to the use of toxic additives such as flame retardants, hardeners, heavy metals (such as lead, copper, and cadmium), phthalates, BPA, and others during plastic production, microplastics may transport contaminants to living organisms and consequently cause bioaccumulation in these organisms (Yurtsever, 2018; Yurtsever, 2019; Önder, 2023; Gençağ, 2025). In some fish species (*Carassius carassius*, *Oreochromis niloticus*, *Danio rerio*, *Oryzias latipes*), nanoplastics ranging in size from 50 to 180 nm have been detected to cross into red blood cells, and it has been reported that they may reach the brain through the bloodstream. They have also been found to inhibit certain enzymes and cause alterations in hematological and hematochemical parameters (Önder, 2023).

7. PLASTIC AND HUMAN HEALTH

Since the 20th century, plastics have begun to pose a threat to all living organisms, particularly humans. Today, plastic use has become so deeply integrated into social life that it has become almost impossible to imagine a world without plastics. Moreover, plastic particles have become so small that people may be unaware that they are consuming plastic. Functional studies on the harmful effects of plastics in humans are very limited. Their presumed effects can be estimated from vertebrate animal models. Micro/nanoscale polymers, additives, and contaminants enter the organism through the digestive and respiratory systems or through epidermal injuries. However, dermal entry remains controversial. Nanoplastics are thought to activate resident immune cells after binding to intestinal or pulmonary surfaces (inner alveolar walls),

potentially causing local inflammatory reactions, damaging cells, and disrupting the composition and functionality of the intestinal microbiome. At the same time, they act as vectors because their surface properties facilitate the transport of pathogenic bacteria (Yurtsever, 2018; WHO, 2019; Kayan & Küçük, 2020). The pathways through which nanoplastics enter the human body are shown in Fig. 11.

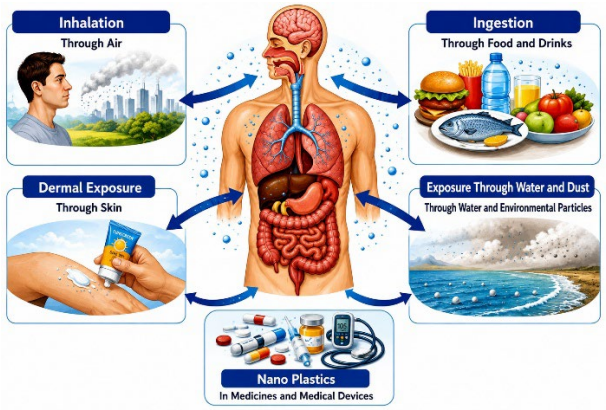

Figure 11. Routes for the entry of micro/nanoplastics into the human body (utilizing artificial intelligence tools)

The detection of microplastics in human blood and tissues, including their presence even in lung tissue, indicates that the issue has reached serious dimensions not only from an environmental perspective but also in terms of public health. In one study, measurable PET, PE, PS, and PMMA polymers were detected in 77% (17 individuals) of 22 blood samples. The average concentration of these particles was reported to be 1.6 µg/mL, and their entry into the body may have occurred through ingestion, dermal exposure, or inhalation. It has also been reported that nanoparticles may be absorbed through the gastrointestinal tract and accumulate in organs such as the brain and liver (Yurtsever, 2018 and 2019; Leslie et al., 2022; Gençağ, 2025). However, the potential effects of long-term exposure are not yet fully understood. Therefore, more detailed studies on this subject are needed (WHO, 2019).

Kayan & Küçük (2020), reported that the toxins contained in plastics may exert carcinogenic effects upon exposure to sunlight, that bread placed in plastic bags while hot may become toxic to humans, and that individuals exposed to micro/nanoplastics over long periods may experience birth defects,

immune system disorders, and developmental delays in children. Plastics do not necessarily have to be in the form of waste to pose a threat to health. Additives used during production can also affect human health. Plastic additives such as BPA (bisphenol A), phthalates, and flame retardants may affect the endocrine system and reproduction. BPA has frequently been detected in urine, blood, and breast milk, as well as in tissues (Yurtsever 2019; Akhan et al., 2022; Gençağ, 2025).

In recent years, microplastics have been detected not only in seafood but also in drinking water, salt, and even the atmosphere. The WHO states that the effects of microplastics on human health are not yet fully understood but that they pose potential risks. Potential effects include oxidative stress, cellular inflammation, endocrine system disruption, and the transport of toxic chemicals (WHO, 2019; Yurtsever 2019; Gençağ, 2025).

8. WHAT CAN BE DONE TO PREVENT PLASTIC POLLUTION?

Plastic pollution is a major global problem that currently poses a significant threat to the environment. Its prevention or reduction requires not only changes in individual consumption habits but also the adoption of a *holistic approach encompassing production, consumption, waste management, and government policies*. UNEP (2023) states that plastic pollution cannot be addressed through recycling alone and that complementary measures are needed, including reducing unnecessary plastic use, developing reuse systems, redesigning products, and increasing recycling capacity. The persistence of plastics in the environment, their accumulation in ecosystems, and their significant environmental impacts throughout the process from production to disposal demonstrate that the problem should not be reduced solely to waste management. According to the OECD, if current policies continue, the amount of plastic waste is projected to triple by 2060 (OECD, 2022b). In this context, *plastic use should primarily be reduced at the source*. In particular, reducing the use of single-use plastic products, plastic bags, and packaging, and promoting the widespread use of reusable products are of great importance

Another important aspect of preventing pollution is the *adoption of a circular economy approach*. The linear economic model (produce–consume–dispose) is one of the main causes of the plastic crisis. Instead of this model, a

circular economy approach based on extending the useful life of products, repairing, refilling, and recycling them should be established (OECD, 2022a). The economic costs of plastic waste in the oceans to wastewater treatment, fisheries, and tourism amount to billions of dollars (UNEP, 2023). As a solution to the problems associated with the synthesis of plastic materials as petroleum-derived polymers and their very long degradation times in the environment, bioplastics are proposed for the production of plastic materials. Bioplastics are alternative materials derived from renewable biological resources such as vegetable oils, starches, or microorganisms, which degrade naturally over time and can be recycled within a biological cycle in terms of their use (Kayan & Küçük, 2020)

Another requirement is *effective waste management and strengthening recycling infrastructure*. The separate collection of plastic waste at the source, its proper sorting, and its delivery to recycling facilities can reduce the leakage of plastics into the environment. However, recycling should not be regarded as a stand-alone solution to plastic pollution (OECD, 2022b). UNEP (2023) reported that only approximately 9% of plastics produced today are recycled and that recycling policies, reducing plastic use, and implementing reuse systems should be applied together. Today, pollution caused by plastic waste associated with increasing plastic production and the need to eliminate such waste have led to the problem of storing or disposing of hazardous/plastic waste, resulting in the widespread expansion of waste trade in underdeveloped and developing countries. Recycling plastics can not only reduce plastic pollution but also provide 80–90% energy savings. In this regard, bioplastic materials derived from renewable resources are reported to help substantially reduce the environmental impacts associated with energy consumption (Kayan & Küçük, 2020; Sifiratik, 2026; Ocak, 2026).

Plastic pollution cannot be reduced through consumer responsibility alone. It is important to implement *policies that increase the environmental responsibility of producers*. By extending producers' responsibilities, they can be required to contribute to the financing or organization of waste management for the products they place on the market after their use. In addition, requirements for the use of recycled plastics, plastic packaging taxes, and regulations targeting single-use plastics are among the economic instruments that can influence production and consumption behaviors (OECD, 2022a). It is

necessary to end business models based on disposable products and single-use packaging and to establish alternative distribution systems centered on reuse and refill (AKD, 2026). In fact, as with all forms of pollution, applying the *"polluter pays principle"* to plastic pollution would be one of the strictest and most effective approaches. Therefore, it is highly important for stakeholder groups such as producers, importers, suppliers, and brand owners to assume greater responsibility for the collection, recycling, reuse, and management of plastic waste. Establishing sustainably managed waste separation bins and recyclable plastic collection centers would also make a significant contribution to combating plastic pollution (Kayan & Küçük, 2020).

Additionally, *transforming consumer behavior and expanding environmental education* are also important. The influence of individual demand on the widespread use of plastics is evident. Therefore, education and awareness-raising regarding the harmful effects of plastics are necessary, and for individual behaviors to become permanent, consumers must be able to access these products economically, and public policies supporting this process must be in place. To prevent microplastic pollution in aquatic environments, the addition of an ultrafiltration unit to wastewater treatment plants and the installation of a unit in washing machines to capture synthetic textile fibers should be considered (Yurtsever, 2018; Kayan & Küçük, 2020; Akhan et al., 2022; Çelebi & Gökmen, 2025; AKD, 2026; Ocak, 2026).

Plastic pollution should be addressed through *international cooperation*. Since plastic waste can be transported from one country to another and seas and oceans encompass the boundaries of many countries, measures taken at the national level remain ineffective in solving the problem (OECD, 2022b). In this context, in 1989, the United Nations (UN) adopted the *Basel Convention on the Control of Transboundary Movements of Hazardous Wastes and Their Disposal*, which regulates the international movement of waste, and it entered into force in 1992. The purpose of the Convention is to eliminate the risks associated with the transboundary movement and disposal of hazardous waste and those arising from recycling. Türkiye signed the Convention in 1989 and became a Party in 1994. As of 2018, 187 countries had become Parties to the Convention, which the United States has not signed to date. However, the global trade in plastic waste has still not been halted. Until 2018, plastic waste trade was primarily conducted from Western countries to China, while Türkiye

became one of the new destinations following China's ban on plastic waste imports in 2018. Türkiye's waste imports increased from 262,000 tons in 2018 to 773,000 tons in 2020. Approximately 30–35% of the imported plastic waste consists of waste that cannot be recycled and therefore requires disposal. This situation contributes to the increasing plastic pollution in Türkiye (Kayan & Küçük, 2020; Gündoğdu & Walker, 2021). In Türkiye, activities aimed at preventing plastic pollution were initiated through the Zero Waste Project launched in 2017 (SıfırAtık, 2026). As of 2018, the use of microbeads added to cosmetics had been banned in almost all countries, particularly the United States, the United Kingdom, Canada, and Australia. Legislative efforts on this issue in Türkiye are also nearing completion (Ministry of Trade, 2026). The *Zero Waste Project in Türkiye and around the World*, led by Türkiye and supported by the UN, together with projects and legislation implemented by the Ministry of Environment and Climate in recent years, such as the DOA (Deposit-Return Packaging) system to be implemented in 2026, as well as initiatives undertaken by other countries and globally active organizations (FAO, UN, etc.), encourage the development of solutions to prevent accumulation in marine ecosystems and to combat pollution (SıfırAtık, 2026).

In conclusion, mitigating plastic pollution requires a holistic approach that incorporates the principles of *"reducing plastic use, reusing, recycling, and shifting toward sustainable alternatives"* collectively. Since reducing the problem solely to consumer behavior would be insufficient, coordinated policies should be developed among producers, consumers, local authorities, and central governments (UNEP, 2023). With increasing awareness since 1991, as of 2018, plastic use had been banned in 51 countries, while plastic bags were subject to taxation in 39 different countries.

Because single-use (disposable) products are the most prevalent issue in plastic pollution, alternatives to these products should be developed urgently, and their use should be rapidly banned

So, why should single-use plastic products be banned?

- Single-use plastics are materials that are consumed excessively due to their low cost and convenience.
- These products, which are manufactured in various types and forms to serve a wide range of purposes, are sold everywhere.
- They become waste immediately after use and are easily dispersed

into the environment.

- They undergo fragmentation through various processes and are transformed into nano- and microplastics.
- They have led to the development of a culture of disposable use.
- They are a major source of nano/microplastics found in bottom sediments and cause significant damage to aquatic ecosystems.
- They are considered to contribute to global climate change (Yurtsever, 2018)

9. EXAMPLES OF PLASTIC POLLUTION STUDIES FROM THE LAKE VAN BASIN AND TURKEY

Atıcı et al. (2020), investigated the general level of pollution associated with plastic debris, based on the Clean Coast Index (CCI), in July 2019 along the section of the Van Yuzuncu Yil University campus located on the shore of Lake Van. Plastic pieces larger than 2 cm were collected along the shoreline, and a total of 1,782 plastic pieces were counted, with a total weight of 20,502.70 g. The total plastic density along the shoreline was 3.56 items/m² (41.01 g/m²), while the CCI value of the shoreline was calculated as 71.28. According to the CCI, the eastern shore of the Zeve Campus was classified as extremely polluted (>20) in terms of plastic debris. In addition, during the collection of plastic pieces, it was observed that the plastics fragmented into smaller pieces and that these fragments entered the lake water

Atıcı et al. (2021), reported that Lake Van, the world's third-largest closed and saline lake (pH 9.8 and salinity 22‰), is a water resource threatened by microplastic (MP) pollution. The only commercial fish species inhabiting the lake is the endemic, anadromous cyprinid *Alburnus tarichi*, known as tarek. Plastic pollution may occur due to wastewater treatment facilities serving the population of Van city as well as the use of monofilament fishing nets and ropes as the main fishing gear. In this study, the presence, frequency, abundance, and types of microplastics in the digestive systems of 101 commercially caught tarek from different regions of Lake Van between January and April 2020 were documented for the first time. The results revealed high levels of microplastics in tarek. A total of 3,338 plastic particles were recovered from the digestive systems of the fish, ranging from 8 to 124 particles per fish (mean: 34±13). The majority of the ingested particles consisted of fibers (74%), while blue-colored

material was the most frequently ingested type of microplastic. The polymers identified by FTIR were polyethylene (PE) and polypropylene (PP). The study showed that the vast majority of tarek were contaminated with microplastics and that the prevalence was higher compared with other studies. Therefore, tarek was reported to be a useful bioindicator of microplastic pollution in Lake Van, and potential human health risks associated with microplastic pollution in Lake Van and the consumption of tarek should be carefully considered.

Atıcı et al. (2022), investigated microplastic pollution levels in the Gulf of Van by collecting samples from 15 stations using a manta trawl with a 335 µm mesh size in November 2019. Microplastics were detected in surface waters at concentrations ranging from 641,424 to 1,426,638 items/km² and from 2.35 to 5.09 items/m³. The most frequently observed type of microplastic was fiber (40.5%), the most common shape was irregular (40.8%), and the predominant color was blue (57.1%). The highest microplastic abundance was observed at stations 14 and 15, where lake currents were influential, and at station 6, which was affected by the Akköprü and Kurubaş streams flowing through the densely populated city center. The chemical composition of the analyzed microplastics was determined as polyethylene (60.2%), polypropylene (20.4%), polyethylene terephthalate (17.2%), and poly (methyl acrylate) (2.2%). As a result, it was demonstrated that the surface waters of the Gulf of Van are contaminated with high levels of microplastics.

Çelik (2026), investigated the presence of microplastics (MPs) in fecal accumulations of the migratory waterbird graylag goose (*Anser anser*), which spends the winter months at Lake Erçek, a closed saline lake in the Lake Van Basin. Samples were collected using a non-invasive sampling method over five periods between October 2024 and February 2025. Suspected MPs in 400 fecal accumulations were examined at the composite period level, with their types, shapes, sizes, and colors determined, polymers identified using FT-IR, and particle surfaces examined using SEM-EDX. As a result, 1.75-2.85 MPs per fecal accumulation (mean: 2.40) were detected. MP abundances peaked in late autumn-early winter and decreased toward the end of winter. Fibers (37.22%), fragments (30.55%), and pellets (18.77%) were the most abundant forms, while the most frequently observed size range was 100-300 µm (30.25%), and the most common color was white/transparent (38.52%). FTIR analyses identified polystyrene, polyethylene, and polyvinyl chloride polymers, while SEM-EDX

detected weathered polymeric surfaces.

Gündoğdu and Çevik (2019a) investigated meso- and macroplastic pollution (0.5-123.4 cm) in 13 coastal areas of the Gulf of İskenderun in May 2018. A total of 1,424 meso/macroplastic items were collected and classified into five categories (filaments, films, foams, fragments, and pellets). The mean data was determined as 12.2±3.5 items/m², with a mean size of 3.7±0.16 cm. Based on their sources, rigid plastics (broken, fragmented, and deformed) were the predominant type, accounting for 59.8%, followed by greenhouse covering films at 11%. In particular, inadequate waste management practices were found to cause coastal ecosystems to become accumulation areas for waste.

Gündoğdu and Çevik (2019b) investigated microplastics in the digestive systems of mullet, horse mackerel, striped seabream, red mullet, red shrimp, and red mullet fish collected from the Mediterranean, Aegean, and Marmara seas, as well as in stuffed mussels consumed in cities such as Ankara, Bodrum, Adana, İzmir, and İstanbul. Microplastics were observed in 63% of red mullet, 64.8% of mullet, 32.8% of red mullet, 34.3% of striped seabream, and 26.7% of horse mackerel, with microplastics detected in 44.3% of all fish examined. The number of microplastics per fish in the seas was determined as 0.85 items in the Marmara Sea, 1.7 items in the Aegean Sea, and 0.74 items in the Mediterranean Sea. Among the red shrimp samples collected from Mersin, microplastics were detected in 18.8% of individuals, with 0.28 items per individual. Microplastics were detected in 91.2% of the stuffed mussels examined, with 0.63 items per individual. Although stuffed mussels are a widely consumed food, when calculated per serving, it was estimated that consuming 100 g of mussels could result in the intake of 5.76 microplastic particles, while consuming 250 g could result in the intake of 14.41 microplastic particles.

Gündoğdu and Walker (2021), in their study highlighting Türkiye's plastic waste imports, reported that following China's ban on plastic waste imports on January 1, 2018, Türkiye became a major importer of global plastic waste. Before the ban, Türkiye imported only 261,864 tons of plastic waste annually, whereas this figure increased to 772,831 tons in 2020, for which USD 143 million was paid. Although Türkiye has recently introduced restrictions on plastic waste imports, illegal dumping and burning of waste have been widely reported. Türkiye ranks second in Europe and seventh worldwide in plastic

production; however, existing domestic waste management and recycling programs remain inadequate in relation to the amount of domestic plastic waste generated. Due to inadequate management of plastic waste, plastics are discharged into the Mediterranean Sea, resulting in Türkiye having the largest share (16.8%) of plastic pollution in European seas.

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CHAPTER 2

PERSONAL CARE PRODUCTS AND THEIR SUBLETHAL AND LETHAL EFFECTS ON AQUATIC ORGANISMS

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1. INTRODUCTION

Personal care products (PCPs) are widely used to maintain personal hygiene, enhance aesthetic appearance, and improve overall quality of life. The rapid increase in the consumption of these products has substantially elevated the likelihood that their chemical constituents will be released into the environment. Together with pharmaceuticals, personal care products are classified as emerging contaminants, representing an important group of pollutants that have become a major focus of research in the protection and management of aquatic environments (Daughton and Ternes, 1999). One of the primary pathways through which PCPs enter the environment is domestic wastewater. During routine activities such as bathing, handwashing, cosmetic removal, and other daily hygiene practices, numerous chemical ingredients are discharged into municipal sewer systems. Although wastewater treatment plants efficiently remove many conventional pollutants, they often fail to completely eliminate certain micropollutants because of their low environmental concentrations, complex chemical structures, and resistance to degradation (Ternes and Joss, 2006; Yang et al., 2017). Following wastewater treatment, residual constituents of PCPs may be discharged into receiving aquatic environments, where they remain dissolved in the water column, adsorb onto suspended particles, or accumulate within bottom sediments. Consequently, aquatic organisms are subjected to continuous or repeated exposure to these contaminants throughout their life cycles (Hernando et al., 2006). Traditional toxicological studies have primarily focused on the acute effects induced by relatively high chemical concentrations. However, environmental toxicology has increasingly recognized the ecological significance of long-term exposure to environmentally relevant concentrations. Even when present at levels far below those causing acute mortality, many ingredients of PCPs can interfere with essential biological processes and adversely affect organismal health (Santos et al., 2010). Aquatic organisms are particularly vulnerable to these contaminants because they spend most or all of their lives in direct contact with the aquatic environment. Numerous studies involving fish, crustaceans, and algae have demonstrated that chemicals originating from PCPs can induce oxidative stress, endocrine disruption, behavioral abnormalities, and metabolic dysfunction (Fent et al., 2006; Wang et al., 2026). Chemicals possessing endocrine-disrupting properties are of

particular concern because they may interfere with hormonal regulation even at extremely low concentrations, ultimately impairing reproductive success and population sustainability. Such effects extend beyond individual organisms and may have profound ecological consequences at both population and ecosystem levels (Gatidou et al., 2007).

The environmental impacts of personal care product-derived contaminants can generally be classified into two major categories. The first comprises lethal effects, which result from relatively high concentrations and short-term exposure leading to organismal mortality. The second includes sublethal effects, in which exposed organisms survive but experience physiological, biochemical, molecular, or behavioral alterations that compromise their fitness. Recent research indicates that these sublethal effects may represent the greatest ecological threat because of their long-term consequences for natural ecosystems (Brausch and Rand, 2011). Therefore, assessing the environmental risks associated with PCPs requires more than simply determining their occurrence in aquatic environments. A comprehensive evaluation should also consider their toxicological mechanisms, biological responses, and ecological consequences in aquatic organisms. Accordingly, this chapter examines the major classes of PCPs ingredients, their pathways into aquatic ecosystems, their environmental fate, and both their lethal and sublethal effects on aquatic organisms from an ecotoxicological perspective.

2. Environmental Sources and Behavior of Personal Care Products in Aquatic Systems

PCPs consist of complex formulations containing numerous active ingredients and auxiliary chemicals. These compounds are incorporated into formulations to improve product stability, extend shelf life, prevent microbial contamination, impart fragrance, or provide specific functional properties. However, following consumer use, a considerable proportion of these substances enters the environment, where some may persist in aquatic ecosystems and exert toxic effects on non-target organisms (Daughton and Ternes, 1999; Caliman and Gavrilescu, 2009; Cui et al., 2025). The environmental fate of chemicals contained in PCPs depends largely on their physicochemical properties, including molecular weight, water solubility, lipophilicity, biodegradability, adsorption capacity, and chemical stability.

While certain compounds remain dissolved in the water column, others readily adsorb onto suspended particles or sediments, or accumulate within biological tissues, thereby increasing their environmental persistence and ecological significance (Hernando et al., 2006; Cui et al., 2025).

2.1. Pathways of Personal Care Products into the Aquatic Environment

Domestic use represents the principal source of PCPs-derived contaminants entering aquatic environments. Products such as shampoos, shower gels, soaps, toothpaste, sunscreens, cosmetics, and perfumes are routinely discharged into municipal wastewater systems following everyday personal hygiene practices. Wastewater treatment plants serve as an important intermediate barrier between domestic wastewater and natural aquatic ecosystems. Nevertheless, many constituents of PCPs are not completely removed during conventional treatment processes because they occur at trace concentrations and possess chemically stable or structurally complex characteristics (Ternes and Joss, 2006; Ankley et al., 2007).

Major classes of PCPs contaminants frequently detected in treated wastewater include:

- UV filters
- Parabens
- Triclosan
- Synthetic fragrance compounds
- Surfactants

(Brausch and Rand, 2011)

In addition to wastewater discharge, recreational swimming activities constitute another important pathway for environmental contamination, particularly in coastal regions. Sunscreen ingredients may be released directly into aquatic environments during swimming, resulting in elevated concentrations of UV filters in popular recreational and tourist areas (Cuderman and Heath, 2007).

2.2. Major Chemical Constituents of Personal Care Products

2.2.1. UV Filters

UV filters are widely incorporated into sunscreen formulations to protect the skin against harmful ultraviolet (UV) radiation. They are also commonly present in moisturizers, cosmetics, and various other PCPs to improve photostability and provide additional UV protection.

Frequently used organic UV filters include:

- Oxybenzone (Benzophenone-3)
- Octinoxate
- Octocrylene
- Avobenzone

These compounds are considered environmentally significant because of their extensive global use, continuous release into aquatic environments, and potential to interfere with biological processes in aquatic organisms (Prichard and Granek, 2016; Srain et al., 2021). The occurrence of UV filters is particularly important in coastal ecosystems, where direct release from sunscreen applications during recreational activities and indirect discharge through wastewater contribute substantially to environmental contamination (Cuderman and Heath, 2007).

Several studies have demonstrated that certain UV filters can:

- induce oxidative stress,
- disrupt endocrine function,
- impair reproductive performance, and
- interfere with developmental processes

(Srain et al., 2021).

2.2.2. Parabens

Parabens are antimicrobial preservatives widely used to inhibit microbial growth in personal care products. The most commonly used derivatives include methylparaben, ethylparaben, propylparaben, and butylparaben. Environmental concern regarding parabens primarily arises from their estrogenic activity. Because several parabens are capable of interacting with estrogen receptors, they are widely recognized as endocrine-disrupting chemicals (Caliman and Gavrilescu, 2009).

Exposure to parabens in aquatic environments may lead to:

- disruption of hormonal regulation in fish,
- impaired reproductive processes,
- altered developmental pathways.

The environmental behavior of parabens depends on their molecular structure. Short-chain parabens generally exhibit higher water solubility and mobility, whereas long-chain derivatives are more hydrophobic and therefore display a greater tendency to accumulate in sediments and biological tissues (Brausch and Rand, 2011).

2.2.3. Triclosan and Other Antimicrobial Compounds

Triclosan is a synthetic antimicrobial compound that has historically been incorporated into numerous PCPs because of its broad-spectrum antibacterial properties. It has been extensively used in soaps, toothpaste, deodorants, and various hygiene products.

From an environmental perspective, triclosan is characterized by:

- relatively low biodegradability,
- pronounced toxicity toward aquatic organisms,
- the potential to alter microbial community composition.

Exposure of aquatic organisms to triclosan has been associated with:

- oxidative stress,
- inhibition of algal growth,
- metabolic disturbances,
- endocrine-related effects

(Gonzalez-Doncel et al., 2020).

Furthermore, triclosan and related antimicrobial compounds are considered potential contributors to the development of antimicrobial resistance in environmental microbial communities. Consequently, these substances represent not only an ecological concern but also an emerging issue for public health.

2.2.4. Surfactants

Surfactants constitute one of the primary functional ingredients of PCPs, providing cleansing, emulsifying, and foaming properties. They are extensively used in shampoos, shower gels, facial cleansers, and numerous household hygiene products.

Surfactants are generally classified into three major categories:

- Anionic surfactants
- Cationic surfactants
- Nonionic surfactants

Their toxicological effects are primarily associated with interactions with cellular membranes. Fish gills are particularly susceptible because of their delicate epithelial structure and direct contact with the surrounding aquatic environment.

Exposure to surfactants may result in:

- damage to gill epithelial tissues,
- disruption of ion regulation,
- reduced respiratory efficiency

(Ahel et al., 1994).

2.2.5. Synthetic Fragrance Compounds

Synthetic fragrance compounds represent another major class of contaminants originating from PCPs. Synthetic musks, in particular, have attracted considerable environmental attention because of their persistence and hydrophobic nature.

These compounds may:

- accumulate in sediments,
- bioaccumulate within aquatic organisms,
- be transferred through aquatic food webs.

Long-term exposure to synthetic fragrance compounds has been associated with metabolic stress and alterations in normal biological functions of aquatic organisms (Daughton and Ternes, 1999).

2.3. Transport and Environmental Fate in Aquatic Systems

Once released into aquatic environments, chemicals originating from PCPs undergo various physical, chemical, and biological transformation processes. These processes largely determine their persistence, mobility, bioavailability, and ecological risk.

The principal environmental processes include:

Photodegradation – degradation of chemical compounds through exposure to solar radiation.

Biodegradation – microbial decomposition of contaminants by naturally occurring microorganisms.

Adsorption – attachment of chemical compounds to sediments or suspended particulate matter.

Bioaccumulation – progressive accumulation of contaminants within biological tissues over time.

While certain PCPs ingredients degrade relatively rapidly, others exhibit considerable environmental persistence. Consequently, ecological risk assessments should consider not only measured environmental concentrations but also the persistence, transformation behavior, and bioaccumulation potential of these contaminants in aquatic ecosystems (Hernando et al., 2006).

3. Lethal Effects of Personal Care Products on Aquatic Organisms

The toxic effects of chemicals originating from PCPs on aquatic organisms vary according to both exposure concentration and duration. Among these effects, lethal toxicity represents the most direct and readily observable endpoint, resulting in organismal mortality. Lethal effects are primarily evaluated through acute toxicity studies and constitute fundamental parameters for determining the environmental hazard potential of chemical contaminants (Fent et al., 2006). In aquatic toxicology, the toxicity of chemical substances is commonly quantified using LC_{50} (Median Lethal Concentration) and EC_{50} (Median Effective Concentration) values. LC_{50} represents the concentration that causes mortality in 50% of the exposed test organisms within a specified exposure period, whereas EC_{50} refers to the concentration producing a defined biological effect in 50% of the exposed population. These indices provide standardized measures for comparing toxic potency among different contaminants and are widely applied in ecological risk assessment (Santos et al., 2010). Although most personal care product ingredients occur at relatively low environmental concentrations, their continuous release, extensive consumption, and inherent biological activity suggest that their acute toxic potential should not be underestimated. In environmentally sensitive species or areas receiving concentrated pollutant inputs, these compounds may induce significant lethal effects (Kumar et al., 2010; Brausch and Rand, 2011).

3.1. Lethal Effects in Fish

Fish are among the most frequently employed vertebrate models in aquatic ecotoxicology because they occupy different trophic levels, exhibit high sensitivity to environmental contaminants, and remain continuously exposed to waterborne pollutants throughout their lives. Toxic effects associated with PCPs ingredients are commonly evaluated through alterations in critical organs such as the gills, liver, kidneys, and nervous system (Fent et al., 2006). Because fish gills are continuously exposed to the surrounding aquatic environment and possess an extensive respiratory surface area, they represent one of the primary targets for chemical uptake and toxicity.

3.1.1. Gill Damage and Respiratory Failure

Surfactants, certain UV filters, and antimicrobial compounds have been reported to induce structural and functional damage to fish gills. Such alterations may result in:

- reduced oxygen uptake,
- impaired ion regulation,
- respiratory distress,
- disruption of energy metabolism.

Surfactants are particularly harmful because they interact directly with cellular membranes, increasing membrane permeability and causing damage to gill epithelial cells (Ahel et al., 1994). Severe gill injury compromises respiratory efficiency and osmoregulatory capacity, ultimately reducing the ability of fish to survive under natural environmental conditions and, in extreme cases, leading to mortality.

3.1.2. Acute Toxicity of UV Filters in Fish

Organic UV filters widely used in sunscreen formulations are increasingly recognized as important aquatic contaminants, particularly in coastal ecosystems. Their direct release during recreational activities and their biologically active nature contribute substantially to their ecological relevance (Cuderman and Heath, 2007).

Acute exposure to UV filters has been associated with several adverse responses in fish, including:

- loss of equilibrium,

- impaired swimming coordination,
- altered respiratory behavior,
- reduced locomotor activity.

Because several UV filters are highly lipophilic, they may accumulate within fish tissues, thereby increasing internal exposure and toxic potential. Metabolically active organs, particularly the liver, are especially susceptible, where contaminant accumulation may trigger detoxification responses and metabolic stress (Gonzalez-Doncel et al., 2020).

3.2. Lethal Effects in Crustaceans

Crustaceans represent ecologically important organisms and are among the most sensitive invertebrate models used in aquatic toxicity testing. In particular, *Daphnia magna* has become one of the internationally accepted standard organisms for evaluating chemical toxicity.

The widespread use of *Daphnia* species is attributed to several favorable biological characteristics, including:

- a short life cycle,
- ease of laboratory culture,
- high sensitivity to environmental pollutants.

Exposure to chemicals originating from PCPs has been shown to induce:

- immobilization,
- reduced swimming performance,
- impaired feeding behavior,
- decreased survival rates

(Brausch and Rand, 2011).

3.2.1. Toxic Effects on *Daphnia magna*

Acute toxicity studies have demonstrated that numerous PCPs ingredients significantly inhibit the swimming activity of *Daphnia magna*. Locomotion is a critical biological function because it directly influences feeding efficiency, predator avoidance, and overall survival.

Reduced mobility may consequently lead to:

- increased energetic costs,
- decreased food acquisition,
- greater vulnerability to predation.

Therefore, toxic effects observed in *Daphnia* populations have ecological implications extending beyond individual organisms, potentially influencing trophic interactions and ecosystem functioning.

3.3. Lethal Effects in Algae

Algae constitute the primary producers of aquatic ecosystems and therefore play an indispensable role in maintaining ecosystem productivity and oxygen generation. Alterations in algal communities may consequently affect the structure and functioning of entire aquatic food webs.

Several PCPs-derived contaminants have been reported to cause:

- inhibition of cellular growth,
- suppression of photosynthetic activity,
- reductions in chlorophyll synthesis,
- population decline resulting from cell mortality

(Gonzalez-Doncel et al., 2020).

Among these contaminants, triclosan has received particular attention because its antimicrobial properties interfere with essential metabolic pathways in algal cells, resulting in pronounced toxic effects.

3.4. Lethal Effects During Early Life Stages

Embryonic and larval stages represent the most vulnerable phases of aquatic organisms with respect to chemical exposure. During these developmental periods, physiological defense mechanisms and detoxification systems remain incompletely developed, rendering organisms particularly susceptible to toxicants.

Exposure to PCPs-derived chemicals during early development may result in:

- arrested embryonic development,
- increased larval mortality,
- developmental abnormalities.

Consequently, environmental risk assessments should include early life-stage toxicity in addition to evaluations conducted on adult organisms, as developmental sensitivity often determines long-term population viability (Fent et al., 2006).

3.5. Ecological Significance of Lethal Effects

Acute mortality caused by chemical contaminants may directly reduce population abundance, particularly in locations experiencing relatively high contaminant concentrations. However, because PCPs residues generally occur at environmentally relevant trace levels, chronic and sublethal effects are more frequently encountered under natural conditions.

Nevertheless, lethal toxicity data remain fundamental for:

- hazard classification of chemical substances,
- development of environmental quality criteria,
- ecological risk assessment and regulatory decision-making

(Santos et al., 2010).

In conclusion, determining the acute lethal toxicity of PCPs ingredients constitutes an essential component of environmental hazard assessment. However, comprehensive protection of aquatic ecosystems also requires consideration of long-term biological responses occurring at sublethal exposure levels, which often exert more persistent ecological consequences than acute mortality alone.

4. Sublethal Effects of Personal Care Products on Aquatic Organisms

The adverse effects of chemicals derived from PCPs are not limited to direct mortality. Because these contaminants are typically detected at relatively low concentrations in aquatic environments, exposed organisms often survive while experiencing physiological, biochemical, molecular, and behavioral alterations. Such responses are collectively referred to as sublethal effects and have become a major focus of modern ecotoxicological research. Although sublethal effects do not immediately result in death, they may substantially reduce organismal fitness and ultimately compromise population sustainability. Decreased reproductive success, impaired growth, suppression of immune function, behavioral abnormalities, and metabolic disturbances are among the most frequently reported consequences of long-term exposure to PCPs-derived contaminants (Fent et al., 2006; Santos et al., 2010). Modern environmental toxicology therefore extends beyond conventional mortality-based endpoints by emphasizing sensitive biological responses that occur well before death. Biomarker analyses, molecular techniques, and behavioral assessments have

become indispensable tools for evaluating the ecological consequences of PCPs contaminants in aquatic ecosystems (Hernando et al., 2006).

4.1. Oxidative Stress and Cellular Damage

Reactive oxygen species (ROS) are naturally produced during normal cellular metabolism in aquatic organisms. Under physiological conditions, ROS are effectively neutralized by endogenous antioxidant defense systems. However, exposure to environmental contaminants may either stimulate excessive ROS production or impair antioxidant capacity, resulting in oxidative stress.

Numerous ingredients found in PCPs are capable of disrupting intracellular redox homeostasis and promoting oxidative damage. Consequently, oxidative stress may induce:

- lipid peroxidation of cellular membranes,
- structural and functional alterations of proteins,
- DNA damage,
- impairment of cellular energy production

(Srain et al., 2021).

Because oxidative stress represents one of the earliest cellular responses to contaminant exposure, it is widely recognized as a fundamental mechanism underlying the toxicity of many PCPs ingredients.

4.1.1. Alterations in Antioxidant Defense Systems

Assessment of antioxidant enzyme activities has become one of the most widely used approaches for evaluating oxidative stress in aquatic organisms.

Among the principal antioxidant enzymes are:

Superoxide Dismutase (SOD): Catalyzes the conversion of superoxide radicals into hydrogen peroxide, thereby protecting cells against oxidative injury.

Catalase (CAT): Converts hydrogen peroxide into water and molecular oxygen, preventing the accumulation of this reactive oxidant.

Glutathione S-Transferase (GST): Plays a central role in detoxification by facilitating the conjugation and elimination of xenobiotic compounds.

Exposure to contaminants originating from PCPs may either increase or suppress the activity of these enzymes, depending on the contaminant, exposure

duration, and species involved. Such enzymatic alterations are widely regarded as reliable biomarkers of chemical stress in aquatic organisms (Gonzalez-Doncel et al., 2020). For example, exposure to triclosan and several organic UV filters has been associated with elevated oxidative stress markers accompanied by significant alterations in antioxidant defense mechanisms in fish.

4.2. Endocrine-Disrupting Effects

Among the environmental concerns associated with PCPs, endocrine disruption has received particular scientific attention. Endocrine-disrupting chemicals (EDCs) are substances capable of interfering with hormone synthesis, transport, metabolism, receptor binding, or hormonal signaling pathways (Kumar et al., 2024).

In aquatic organisms, the endocrine system regulates numerous essential biological processes, including:

- reproduction,
- embryonic development,
- growth,
- metabolism,
- behavior.

Consequently, disruption of hormonal homeostasis may produce profound ecological consequences, particularly in fish populations where reproductive success is highly dependent on endocrine regulation.

4.2.1. Effects on Reproductive Function

Parabens, several organic UV filters, and synthetic fragrance compounds are among the principal personal care product ingredients recognized for their endocrine-disrupting potential.

Exposure to these chemicals has been associated with:

- alterations in estrogenic and androgenic hormone levels,
- impaired gonadal development,
- reduced gamete production,
- abnormal reproductive behavior

(Caliman and Gavrilescu, 2009).

Because successful reproduction is fundamental to population persistence, endocrine-mediated reproductive impairment may ultimately

contribute to declining population sizes and reduced biodiversity within aquatic ecosystems.

4.3. Effects on Growth and Development

Early developmental stages are particularly susceptible to environmental contaminants because physiological defense mechanisms remain incompletely developed during embryonic and larval life.

PCPs-derived chemicals may adversely affect growth and development through:

- disruption of cellular proliferation,
- alteration of energy metabolism,
- delayed developmental progression,
- reduced larval survival.

Experimental studies employing zebrafish (*Danio rerio*) and other aquatic model organisms have demonstrated that several cosmetic ingredients interfere with normal embryonic development and larval growth, emphasizing the vulnerability of early life stages (Fent et al., 2006).

4.4. Behavioral Alterations

Behavioral responses constitute some of the earliest and most sensitive indicators of environmental stress. Consequently, behavioral ecotoxicology has become an increasingly important component of ecological risk assessment.

Exposure to PCPs contaminants may induce:

- altered swimming activity,
- impaired feeding behavior,
- modified avoidance responses,
- increased stress-related behavior.

In fish, locomotor performance is directly linked to essential ecological functions, including food acquisition, predator avoidance, territorial interactions, and reproductive behavior. Therefore, behavioral disturbances observed at the individual level may ultimately translate into population-level consequences.

4.5. Effects on the Immune System

Aquatic organisms continuously encounter pathogens and diverse environmental stressors throughout their life cycle. The immune system therefore plays a critical role in maintaining organismal health and resilience.

Certain constituents of PCPs have been shown to:

- alter immune cell activity,
- modify inflammatory responses,
- reduce resistance to infectious diseases.

Suppression of immune function increases susceptibility to both pathogens and additional environmental stressors, thereby compromising the long-term health and survival of exposed populations (Fent et al., 2006).

4.6. Genotoxic and Molecular Effects

Molecular biomarkers have become indispensable tools for detecting early biological responses to chemical exposure before visible physiological damage becomes apparent. These approaches enable the identification of subtle cellular alterations that cannot be detected using conventional toxicity tests.

Several PCPs ingredients have been associated with:

- DNA damage,
- altered gene expression,
- activation of cellular stress-response pathways.

Commonly applied methods for evaluating genotoxicity include:

- the Comet assay,
- the Micronucleus assay,
- gene expression analyses.

These molecular responses serve as valuable early-warning biomarkers, indicating exposure to environmental contaminants and providing insight into the mechanisms underlying toxic effects.

4.7. Ecological Significance of Sublethal Effects

Although sublethal responses do not immediately cause mortality, they frequently exert far-reaching ecological consequences. Long-term exposure to environmentally relevant concentrations of PCPs contaminants may result in:

- reduced reproductive success,
- decreased population growth rates,

- altered species interactions,
- disruption of ecosystem functioning.

Unlike acute toxicity, which primarily affects individual survival, sublethal effects accumulate over time and may gradually alter population dynamics and ecosystem stability. Consequently, comprehensive environmental risk assessments should integrate chronic toxicity endpoints together with sublethal biological responses to provide a more realistic evaluation of ecological hazards associated with personal care products.

5. Ecological Risk Assessment, Sustainable Approaches, and Future Perspectives

Understanding the impacts of PCPs-derived chemicals on aquatic ecosystems requires more than evaluating the toxicity of individual compounds. Their environmental behavior, exposure pathways, persistence, biological effects, and long-term ecological consequences must be considered collectively. Within this framework, ecological risk assessment (ERA) has become one of the principal approaches for evaluating the environmental hazards associated with PCPs (Hernando et al., 2006; Maghimaa et al., 2026).

Ecological risk assessment generally consists of three fundamental steps:

1. Hazard Identification – Determination of the toxicological properties of a chemical and identification of its mechanisms of action.

2. Exposure Assessment – Evaluation of the concentrations and duration of chemical exposure experienced by aquatic organisms under environmentally relevant conditions.

3. Risk Characterization – Integration of toxicity data with environmental exposure information to estimate the likelihood of adverse ecological effects.

This framework enables researchers and environmental managers to determine not only whether a chemical possesses intrinsic toxicity but also whether it presents an unacceptable ecological risk under real-world environmental conditions (Santos et al., 2010).

5.1. Bioaccumulation and Transfer through the Aquatic Food Web

Following their release into aquatic environments, certain PCPs ingredients may accumulate within the tissues of living organisms. Bioaccumulation refers to the progressive uptake and retention of chemical substances from the surrounding environment over time.

Compounds exhibiting the following characteristics generally present the greatest bioaccumulation potential:

- high lipophilicity,
- environmental persistence,
- limited metabolic degradation.

(Daughton and Ternes, 1999).

As a consequence of bioaccumulation, contaminants occurring at very low concentrations in water may reach substantially higher levels within biological tissues. This phenomenon is particularly significant for long-lived organisms and species occupying higher trophic levels.

Transfer through the aquatic food web typically follows the sequence:

Phytoplankton and algae → Zooplankton → Small fish → Predatory fish → Top consumers

Accordingly, environmental risk assessments should evaluate not only contaminant concentrations in water but also their accumulation potential in aquatic organisms and their movement through food webs (Sabino et al., 2021).

5.2. Ecological Effects of Chemical Mixtures

Under natural environmental conditions, aquatic organisms are rarely exposed to a single contaminant. Instead, they encounter complex mixtures of chemicals derived from PCPs, pharmaceuticals, pesticides, industrial pollutants, and numerous other anthropogenic sources.

The combined effects of chemical mixtures may generally be classified into three categories:

Additive Effects: The overall toxicity corresponds approximately to the sum of the individual effects of each chemical.

Synergistic Effects: Combined exposure produces greater toxicity than expected from the sum of individual chemical effects.

Antagonistic Effects: One chemical reduces or counteracts the toxicity of another.

Although conventional toxicity assessments have traditionally focused on individual compounds, real aquatic ecosystems are characterized by continuous exposure to multiple contaminants. Consequently, future ecotoxicological studies should increasingly emphasize mixture toxicity to improve the ecological relevance of environmental risk assessments (Caliman and Gavrilescu, 2009; Maghimaa et al., 2026).

5.3. Environmental Monitoring and Analytical Approaches

Reliable detection of contaminants originating from PCPs requires highly sensitive analytical techniques because these substances are generally present at trace concentrations.

Among the analytical methods most widely employed are:

- Liquid Chromatography–Tandem Mass Spectrometry (LC–MS/MS),
- Gas Chromatography–Mass Spectrometry (GC–MS),
- High-Resolution Mass Spectrometry (HRMS)

(Wang et al., 2026).

However, chemical monitoring alone cannot adequately characterize ecological risk. Biological monitoring is equally important for evaluating the actual biological consequences of contaminant exposure.

Frequently used biological assessment tools include:

- antioxidant enzyme activities,
- biomarkers of DNA damage,
- gene expression analyses,
- behavioral responses.

The integration of chemical analyses with biological monitoring provides a more comprehensive understanding of contaminant impacts on aquatic ecosystems.

5.4. Wastewater Treatment Technologies for the Removal of Micropollutants

Wastewater treatment systems represent one of the most important barriers preventing the release of PCPs contaminants into natural aquatic environments. Nevertheless, conventional biological treatment processes often

exhibit limited efficiency in removing many persistent micropollutants (Yang et al., 2017; Maghimaa et al., 2026).

To overcome these limitations, several advanced treatment technologies have been developed.

Advanced Oxidation Processes (AOPs): Advanced oxidation technologies—including ozonation, photocatalysis, and related processes—generate highly reactive oxidizing radicals capable of degrading persistent organic contaminants.

Activated Carbon Adsorption: Activated carbon effectively removes numerous organic micropollutants through adsorption and is widely incorporated into advanced water treatment systems.

Membrane Technologies: Membrane filtration techniques provide highly efficient removal of dissolved organic contaminants and represent an increasingly important component of advanced wastewater treatment.

Despite their effectiveness, implementation of these technologies should also consider operational costs, energy consumption, maintenance requirements, and the possible formation of transformation by-products.

5.5. Sustainable Personal Care Products and Green Chemistry

Reducing the environmental impacts of PCPs requires strategies extending beyond improvements in wastewater treatment. Environmental sustainability should be integrated into product design through the principles of green chemistry**.**

Important objectives include:

- replacing hazardous ingredients with less toxic alternatives,
- increasing the use of readily biodegradable compounds,
- minimizing the incorporation of persistent and bioaccumulative chemicals,
- reducing unnecessary chemical additives.

Development of environmentally safer formulations by manufacturers, together with increasing consumer preference for sustainable products, will contribute substantially to the long-term protection of aquatic ecosystems.

5.6. Future Research Priorities

Although considerable progress has been made in understanding the ecological effects of PCPs, significant knowledge gaps remain.

Future research should particularly focus on:

- long-term consequences of chronic low-dose exposure,
- ecological effects of chemical mixtures,
- environmental behavior of emerging cosmetic ingredients,
- interactions between climate change and chemical toxicity,
- population- and ecosystem-level responses to contaminant exposure.

Furthermore, advances in molecular biology, omics technologies, and artificial intelligence-assisted ecological risk assessment are expected to substantially improve our ability to predict, monitor, and mitigate the environmental impacts of PCPs contaminants.

6. Conclusion

PCPs have become indispensable components of modern daily life. However, following their use, many of their chemical constituents are released into aquatic environments, where they represent an important source of emerging micropollutants. Ingredients commonly found in shampoos, cosmetics, sunscreens, fragrances, and personal hygiene products are transported into aquatic ecosystems primarily through wastewater discharges, exposing aquatic organisms to a wide variety of biologically active compounds. Major classes of PCPs ingredients—including UV filters, parabens, triclosan, surfactants, and synthetic fragrance compounds—have been shown to induce both lethal and sublethal effects in aquatic organisms. While acute exposure may result in direct mortality, chronic exposure to environmentally relevant concentrations can produce a broad spectrum of biological responses that are often more ecologically significant. Sublethal effects, including oxidative stress, endocrine disruption, genotoxicity, immunosuppression, behavioral alterations, and reproductive impairment, may initially occur at the level of individual organisms but can progressively influence population dynamics, community structure, and ecosystem functioning. These findings demonstrate that the ecological consequences of PCPs contamination extend well beyond immediate toxic effects and should therefore be evaluated within a broader ecological context. Consequently, environmental risk assessment should not

rely solely on acute toxicity data. Instead, comprehensive evaluations should integrate chronic toxicity, bioaccumulation potential, mixture toxicity, environmental persistence, and ecological interactions in order to provide a more realistic assessment of environmental hazards associated with PCPs. Reducing the environmental burden of these contaminants requires the implementation of sustainable manufacturing strategies, the development of environmentally compatible product formulations, improvements in advanced wastewater treatment technologies, and continuous environmental monitoring programs. Such integrated approaches are essential for minimizing contaminant release and protecting aquatic ecosystems. In conclusion, PCPs should be regarded not only as consumer goods with direct benefits for human health and well-being but also as environmentally relevant contaminants requiring careful ecological consideration. Future research should focus on developing innovative, sustainable, and environmentally benign PCPs that meet consumer needs while minimizing adverse effects on aquatic ecosystems.

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CHAPTER 3

THE ANIMAL IS MORE THAN THE FOOD

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INTRODUCTION

From animal, humans need clothing, shelter, and protection from heat and cold. The raw materials for these needs—skin, wool, and hair—are obtained from these animals. Animals, also, provide many benefits to humans through their reproduction, milk, meat, egg, farming, sale for profit, and rental. Food is another indispensable human need. From this perspective, the benefits animals provide to humans are clear. We benefit from their meat. We eat and live on the products derived from their bodies or services. Human needs are not limited to bodily and physical needs. The spiritual needs of humankind are also compensated with animals, as can be across some people you have seen with animals during their whole domestic life. In the mornings, when they go out to graze, in the evenings when they return home with full bellies and udders, when they meet their young and bleat, and when they run and play as they go out to graze again, there are many beautiful scenes for humans to watch and enjoy. These animals also help us with our transportation needs. Fowls, cattle, sheep and goat carry both us and our loads wherever we want to go. If we were to carry those loads ourselves, how difficult it would be; we would be exhausted, almost to the point of death. And even then, we wouldn't be entirely successful. Above all, these animals perform this service for us without any compliment. Apart from these benefits, animals have ole on human life in different aspects. This chapter is relating on this issue in some how. My field is animal science. My intention is to teach effectively animal science when they grow up in university age. Animals are not breeding for only food but also they ared produced for accompany or different purposes to easy our life. For this reason, it was focused some subject on animals and mankind with different concepts here.

As a Friend

The socially constructed nature of human-animal relationships highlights the power of expectations, beliefs regarding theory of mind, and the need for social belonging in determining human happiness, health, and well-being. Animals can serve as significant resources for humans, fulfilling roles such as companion, helper, therapist, and family member. It is evident that when we attribute capacities like theory of mind and anthropomorphism (the attribution of human characteristics) to animals, we enable them to provide us with

meaningful social support and a sense of purpose in life. As social resources, animals are associated with an impressive array of positive benefits for health and well-being. Reflecting on the nature of human-pet relationships encourages psychologists to revisit fundamental questions such as "What is a friend?" or "What is a family?" After all, in a scenario where a member of a different species can be accepted as part of one's family, the classic attributes found in many definitions of family (such as blood ties) may prove insufficient to capture the essential elements that truly define such a strong "in-group" structure. By examining human-animal interactions, we not only gain insight into how animals influence and enrich our lives but also discover more fundamental truths about key aspects of our own humanity—including the building blocks of friendship, family, and love. Thus, it is hardly surprising that people confide in their pets, take them along on family vacations, and experience profound grief upon their passing. When animals become companions, the bonds they form with humans can be just as deep and meaningful as any other relationship in a person's life (McConnell et al., 2016).

Whether animals—especially domestic animals—can be considered friends depends on how the concepts of both friendship and animals are understood. Certain definitions of friendship may more readily encompass (non-human) animals than others. Regardless of how friendship is defined, viewing animals as (potential) friends could be thought to foster better treatment of them, as various forms of mistreatment or exploitation are incompatible with treating someone as a friend. However, concern for animal welfare typically extends far beyond direct interest in the animals within our own homes to encompass animals (or specific animal species) in general, whereas friendship is inherently particularistic in nature. Consequently, there are limits to applying the concept of friendship to the treatment of animals beyond specific individual relationships (Townley, 2011). Above all, Sconton (2017) argues that humans have an obligation to socialize with domesticated animals—particularly members of farm animal species—by making an effort to include them in their social lives in ways that allow for the formation of friendships. In other words, in addition to (and without prejudice to) their fundamental welfare-based rights to socialize with members of their own and other species, domesticated animals are also entitled to opportunities to form friendships with humans. The reasons for this are as follows: i) domesticated

animals are already involved in a relationship of unjust social cooperation and dependency with humans; and ii) for their interests to be robustly represented and their agency enabled within a just interspecies community, ongoing human moral concern and "social capital"—an indispensable source of personal friendships—are vital. Consequently, I argue that engagement with farm animal sanctuaries offers a promising avenue for fulfilling this obligation and supports approaches that view the sanctuary as a just interspecies community.

As a Teacher

Jane Bone's article, "The animal as fourth educator: A literature review of animals and young children in pedagogical relationships" (Bone, 2013), examines the role of animals in children's development and learning processes. The author argues that animals are significant pedagogical actors in the environment where children live and learn. This idea is developed from Loris Malaguzzi (1998)'s definition of the environment as the "third educator" in the Reggio Emilia approach. According to Bone, after teachers and the environment, animals can also be considered as the "fourth educator," actively playing a role in children's learning processes. Bone (2013) emphasizes that animals contribute to children's social, emotional, cognitive, and ecological development; however, this relationship must be carefully considered in terms of ethics, health, safety, and animal welfare. Humans have lived alongside animals throughout history, and the human-animal relationship has held a significant place in children's lives. Today, environmental problems and the decrease in biodiversity have made children's relationships with animals and nature even more important. According to Wilson's "biophilia" approach (Wilson, 1984), humans have an innate tendency to feel interest and affinity towards living things. Positive relationships that children form with animals can support the development of positive attitudes towards nature and the environment. It has been observed that children who own pets at home exhibit more positive attitudes towards other animals. Research also shows that young children can understand ecological relationships. For example, children can understand that damage to a forest can lead to animals losing their food and shelter resources. This demonstrates that animals can teach children not only biological information but also ecological relationships and the interdependence of life. Live animals can increase children's motivation to

learn. It has been found that children in an environment with a live dog need less guidance, focus more on tasks, and are more willing to learn. Children often perceive animals as non-judgmental, calm, and reassuring beings. These characteristics of animals are particularly important for children who experience stress or need additional support during the learning process. The presence of live animals can increase children's attention, help them calm down, and make it easier for them to participate in learning activities. Therefore, animals are considered not only as objects to be observed but also as elements that actively participate in children's learning process. Children are able to make moral decisions about animals independently of adults. For example, some children may choose not to eat meat because they love animals. This shows that children's developing emotional and moral attitudes towards animals should be considered in terms of education. Animals can teach children not only biological facts but also values such as compassion, empathy, and ethical responsibility. Animal-assisted therapies have shown positive results, especially in children with developmental differences. Research has shown that children are calmer, more sensitive, and more communicative in environments where a live dog is present. Temple Grandin's (Grandin, 2006) experiences also show that contact with animals can create peace and tranquility in people. It is suggested that direct contact with animals can support the development of empathy in children. A child who notices the emotional responses of an animal may become more sensitive to the emotions of other people as well. Animals hold an important place in children's family lives. Children may perceive their pets not just as objects, but as friends, family members, and individuals with whom they form close relationships. Therefore, animals are an important part of children's social world. Children can form strong emotional bonds with animals and see them as family members. Research has shown that children are willing to talk about animals and identify them as individuals, friends, or relatives. The place of animals within the family is also important for educators. The experiences children share about their pets at home can provide information about their emotional and social lives. In addition, research on domestic violence shows that animal abuse may be related to other forms of violence within the family. Therefore, violence against animals is an important issue not only from the perspective of animal welfare but also from the perspective of child development. Children who show love, care, and

responsibility towards animals may develop empathy and positive social behaviors. For animals to truly be the "fourth educator," educators must also show responsibility towards animals. The care, rest, safety, and emotional needs of animals must be taken into consideration. Children imitate animals, imagine themselves as cats, dogs, or other animals, and incorporate the behaviors they learn from animals into their games. These games also contribute to children's understanding of social relationships, power dynamics, and societal roles. The animal acts as a bridge between the child's imagination and reality. Children should be taught how to approach animals, when animals might be frightening, and when they might cause harm. It is important for educators to teach children safe behavior rules in conjunction with animals. In addition, the animals' health checks, vaccinations, hygiene conditions, allergies, and risks of infectious diseases should be considered. Keeping animals under constant adult supervision is necessary for the safety of both children and animals. The need for a more serious and comprehensive approach to animals in early childhood education is crucial. Animals can teach children about biology, ecology, and nature, as well as important values such as empathy, love, responsibility, care, ethics, and safety. However, viewing animals as the “fourth educator” should not mean using them solely as tools to benefit children. Animals also have needs, welfare, and a right to life. Educators should establish relationships based on mutual respect that benefit both children and animals. In summary, Bone (2013) suggests that more sensitive, ethical, and open-to-learning relationships can be established in educational environments where both children and animals live together.

As a Forest Fire Preventer

In fact, the traditional approach creates a scenario where both foresters and hair goat herders benefit. Under this arrangement, the forest administration acknowledges the villagers' grazing needs and allocates suitable forest areas to them for that purpose. In return, the villagers agree to abide by the rules set by the forest administration; consequently, both parties achieve higher yields per unit area, and the risk of forest fires is reduced. Managing combustible materials is the primary strategy proposed to reduce the intensity and impact of major forest fires. Dry herbaceous vegetation, low-hanging tree branches, and accumulated leaf litter and twigs can easily build up—in both forest and

agricultural areas—creating a supply of dry, flammable material; if this material comes into contact with fire for any reason, forest fires can readily ignite. It is suggested that grazing goats on these combustible materials can help prevent forest fires by clearing away some of the undergrowth (Tolunay et al., 2011; Tolunay and Özmiş, 2022). Livestock grazing by nomadic herders in forest areas is viewed as a problem by the Forest Management Directorate in areas undergoing regeneration. However, it can also be considered that grazing in areas with mature trees may reduce fire risk and contribute positively to the ecological balance (Karagöl, 2016). Today, forest fires pose an increasingly significant problem both globally and within our country. Many European countries allocate a substantial portion of their revenues to combating forest fires. Despite heavy investments in ground and aerial assets, equipment, and personnel for firefighting, the incidence of forest fires continues to rise. Beyond existing prevention and intervention techniques, it is necessary to consider alternative approaches and to develop and test additional strategies. One such approach is the use of livestock grazing to remove understory herbaceous and shrubby vegetation, which can otherwise fuel surface fires that may escalate into crown fires. Controlled grazing—conducted at the right time and intensity in suitable areas where grazing poses no ecological risk—can play an effective role in clearing surface fuels (such as low-lying vegetation, woody debris, and shrub layers) that act as combustible material in the forest understory. Reducing understory biomass density, particularly prior to the fire season, not only lowers the risk of potential forest fires but also alters the forest fire regime. Controlled livestock grazing serves as a highly effective complement to existing forest fire prevention techniques. Every year, vast areas of forest are lost to wildfires. In addition to conventional firefighting and prevention efforts, grazing should also be utilized as an alternative measure to help prevent the wildfires that cause such damage. However, it is beneficial for grazing to be conducted under controlled and planned conditions—involving appropriate animal species and numbers—and under the supervision of forest managers. Controlled grazing plans should be developed by forestry authorities, and the feasibility of the practice as an effective and economical solution must be carefully assessed through surveys of the vegetation and terrain in fire-prone areas. Grazing operations must be tailored to the specific plant species and the topography of the area. Furthermore, these activities should be carried out with the assistance

of experienced shepherds who are intimately familiar with the terrain (Bilgili et al., 2017).

Animals In Curing

Animal-assisted therapy has evolved in parallel with the practice of keeping animals as pets since its inception; many of these therapeutic practices represent an extension of the now-recognized health benefits experienced by individuals who own or interact with animals. These benefits range from early observations of lowered blood pressure in those interacting with animals to distinct behaviors indicative of a more relaxed state of mind (Beck, 2000). Cevizci et al. (2009) stated in their review article that animal-assisted therapy aligns with the public health goal—frequently emphasized—of achieving and enhancing the complete physical, social, and mental well-being of individuals. Although questions remain regarding its effectiveness in group therapy versus individual treatment and the ideal temperament and characteristics for participating animals, the temperaments of all animals—regardless of the program type—must be assessed; they must undergo veterinary examinations and receive training in listening and learning before working with patients. In Türkiye, animal-assisted therapy is not practiced as a specialized field; instead, only limited services are offered based on the positive outcomes resulting from human-animal interaction. Furthermore, there are no scientific studies conducted in this field within the country. Cevizci et al. (2009) suggested that when implemented in accordance with guidelines and ethical standards, animal-assisted therapy can serve as a valuable supportive treatment option for improving human health and quality of life, as well as for maintaining health status. According to the manuscript of Çakıcı & Kök (2020), animal-assisted therapy is an alternative treatment method based on human-animal interaction, used alongside primary treatments to improve the quality of life and well-being of individuals suffering from chronic illnesses or affected by mental disorders. It is known to serve as a supportive therapy for a wide range of conditions, including pervasive developmental disorders, cancer, Alzheimer's disease, dementia, feelings of loneliness, anxiety, and depression. Research conducted abroad has yielded positive results when treatments are administered according to established protocols by skilled and competent teams. However, the number of studies conducted in this field in Türkiye remains limited. This review, which

aims to examine animal-assisted therapy from a multifaceted perspective, covers the definition, history, and application areas of the therapy, as well as the animal species involved and its implementation across different life stages, such as childhood and old age. Given the positive outcomes observed in treating various diseases and mental disorders, it is considered beneficial to implement animal-assisted therapy in Türkiye—exploring the use of different animal species and extending its application to a broader range of mental disorders and medical conditions.

In virtually every human culture, animals have served as medical resources for treating and alleviating countless diseases and ailments. Although often dismissed as superstition, the significance of animal-based traditional medicine is undeniable; indeed, pharmaceutical companies have systematically studied these animals as sources of medication for modern medical science. The phenomenon of zootherapy provides compelling evidence for the use of animal-derived resources for medical purposes. In fact, pharmaceutical and agri-food companies have been utilizing animals for decades without making any payments to the countries where these genetic resources originate. The use of animal body parts in folk medicine places additional pressure on wildlife populations that are already in critical condition. Many animal species have been overexploited due to their use as sources of medicine in traditional trade as well. Research into zootherapy must align with the welfare of the animals used for medical purposes, and the utilization of by-products derived from them must be carried out sustainably. Today, there is a growing consensus that sustainability should be adopted as a fundamental principle in biological conservation efforts (Costa-Neto, 2005). Also, Walsh (2009ab) explained the importance of human-animal bonds historically, across cultures, and in recent research. However, attachments with companion animals had been undervalued and even pathologized in the field of mental health. His article briefly surveys the evolution of human-animal bonds, reviews research on their health and mental health benefits, and examines their profound relational significance across the life course. Finally, the emerging field of animal-assisted interventions is described, noting applications in hospital and eldercare settings, in the family and in innovative school, prison, farm, and community programs, to give more attention to these vital bonds in systems-oriented theory, practice, and research. The vast majority of pet owners view their pets as family

members; however, the role of pets within family systems and family therapy has received scant attention in research, training, and practice. It can be said that there are the benefits of pets for family life and family therapy as valuable resources for healing (Walsh, 2009b). It appears that the use of animals in treating human diseases—specifically apitherapy and leech therapy, which represent the most benign or uncontroversial approaches in this field—will increasingly continue within the scope of alternative medicine practices. For agricultural engineers specializing in animal science, playing a more active role in this area is highly significant in terms of professional development and expanding their scope of practice.

Other Roles

Animals are used in narcotic investigations and earthquake prediction. Some evidences are given here:

Fattah et al. (2022) investigated the effects of breeding type, weaning time, and maternal bonding on the narcotic detection effectiveness of police dogs. One hundred German Shepherd dogs (52 males and 48 females) were used in the study. Three trials were conducted. Trial I: The dogs were divided into two groups according to their previous breeding systems; the first group received natural breeding (natural nursing), and the second group received artificial nursing. Trial II: The dogs were divided into three groups. The dogs in the first group were weaned at 8 weeks, the dogs in the second group at 12 weeks, and the dogs in the third group were orphaned. Trial III: The dogs were divided into two groups. The first group consisted of dogs trained with their mothers present, and the second group consisted of dogs trained in the absence of their mothers. Their results showed that the drug detection training performance measures were significantly affected by factors such as nursing method, weaning time, and maternal bonding. Dogs raised in a natural environment, dogs weaned at 12 weeks, and dogs trained with their mothers present performed better, with higher correct warning rates and lower false warning rates. Their observations indicate that past practices (breastfeeding method, extended breastfeeding duration, and maternal bonding) positively influence the future behavior and drug detection effectiveness of police dogs without the need for additional reinforcement.

As with many environmental changes before major earthquakes, changes in animal behavior have also been observed. Before the magnitude 6.3 earthquake that struck L'Aquila, Italy on April 6, 2009, frogs exhibited quite unusual behavior: a few days before the earthquake, the frogs suddenly disappeared from their breeding grounds in a small lake about 75 km from the epicenter and did not return after the series of aftershocks (Grant et al.,2011). Reports have been compiled on anomalous animal behavior related to earthquakes in Colombia covering the period 1610-2019. These reports reveal that for 41 devastating earthquakes, 138 animals, mostly belonging to the Chordata phylum, Mammalia class, Carnivora order, Canidae family, Canis genus, and C. familiaris species, responded to seismic activity. Initial findings indicated that the simultaneous responses of C. familiaris, B. taurus, and E. caballus species were more dominant than their pre- and post-earthquake responses, while the second set of findings showed that pre-earthquake behavior was dominant (Sanchez,2024). The other roles of animals are countless. For example, they protect our property and livestock from predators. Shepherds graze their flocks with the help of dogs.

Empathy & Anthropomorphism

Animal caretakers with high levels of empathy do not view their animals as machines or their farms as factories; even if their relationship with their animals results in economic consequences, they see them as individuals, even giving them names and individualizing them. Kielland et al. (2010) reported that the empathy level of farmers affects human-animal relationships and positively influences the caretakers' behavior towards animals and, consequently, animal welfare. The satisfaction of the animal caretaker with their job affects the welfare and productivity of the animals. When the caretaker's own needs are met while striving to achieve the goals of the enterprise, their work motivation and commitment to their job increase, and the quality of their interactions with animals also improves. This is because the work motivation and commitment of the animal caretaker directly and positively influence other important characteristics related to their job, such as their willingness to acquire new knowledge and skills. Kılıç and Bozkurt (2013) noted that farmers who care for their animals themselves and have high job

satisfaction and motivation also have a high perception of animal welfare (Bozkurt et al., 2013).

The indirect teacher role of animals is clearly seen in their various roles in children's books and cartoons. In picture books, animal images are often anthropomorphic, and visual representations of animals both reflect and create cultural values. It is clear that reading about animals in books contributes to biological knowledge. However, over time, anthropomorphism, meaning attributing human psychological qualities to animals, has begun to spread among people today (Wynne, 2007). In fact, anthropomorphism is very natural in humans. Children associate any self-moving or animal-shaped object with humans. This can also develop in adults. When we approach these people from a consumer perspective, they reject animal products because they attribute human characteristics to animals. If this starts at an early age, nutritional deficiencies and imbalances may arise in individuals. In this case, establishing a boundary between animal and human at an early age can prevent anthropomorphism beforehand. The increasing mistreatment of animals triggers such thoughts. Many children's books are about pets, but rarely are farm animals or animal rights discussed. In recent years, a mindset has begun to develop among young people that goes beyond veganism and focuses on the idea of how we can eat the meat of our friends (Wang and Basso, 2019; Kim ve Yoon, 2021). According to Niemyjska et al. (2018), individuals who do not include meat in their diets are not only free from situational pressures that compel one to sever moral ties with animals or refuse to attribute human-like mental traits to them; they may also be temperamentally more inclined to ascribe human qualities to non-human animals compared to individuals who consume all types of food. In their study, Niemyjska et al. (2018) sought to determine whether individual differences in anthropomorphism were associated with the empathic bond formed with non-human animals and the consequent reduction in meat consumption. Their studies confirmed that reduced meat consumption was linked to both a greater recognition of human-like traits in animals and increased empathy toward them. Most importantly, their data support a model in which the tendency to attribute human traits to animals (anthropomorphism) predicts levels of empathy. Empathy, in turn, leads to reduced meat consumption by heightening the perceived significance of potential harm inflicted upon animals through meat-inclusive dietary

choices. The important thing here is not to give animals a human identity or to constantly empathize with them, but to treat all living things in the ecosystem with human dignity. In the case of domesticated animals, the responsibility for them rests entirely with us; in nature, they could live independently. By domesticating them, we selectively reduced some of their self-defense instincts. Therefore, providing animals with the necessary care, nutrition, comfort, and well-being in return for what they provide us is a fundamental duty of those involved in animal husbandry. Only when this is achieved can consumer perceptions be shaped as they should be.

CONCLUSION

Today, animals are constantly the focus of attention as creatures that are disappearing, threatened, endangered, suffering, imprisoned, killed, sterilized, pampered, trained, wild, predatory, exploited, loved, hunted, cared for, sick, controlled, domesticated, and protected. Yet, animals provide humanity with food, transportation, clothing, companionship as pets, income by participating in races on our behalf, and are sacrificed and offered as a token of our gratitude to God. Furthermore, wool, which should be used in the clothing industry, is being researched for its potential use as insulation material in the construction sector, and lanolin, a component of wool, is a highly sought-after ingredient in the cosmetics industry, demonstrating that animals' benefits to humankind go beyond food. In addition to all this, animals are the fourth teacher for children, predict earthquakes, provide healing through their products and themselves, are friends, catch criminals, and even, at one time, carried out suicide attacks in place of soldiers. And there are countless other characteristics we have yet to discover or understand. As a human beings, we need to reconsider our perspective on animals, our teachings on how we should treat these creatures, these gifts from God, according to their species. Otherwise, since humans, being omnivores, inevitably need animal-derived foods to meet their nutritional needs, instead of rejecting the products animals offer us by attributing human identities to them, our most important stance should be to treat them humanely, ethically and with animal welfare in mind, regardless of their species.

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CHAPTER 4

BEHAVIOR MONITORING IN CHICKENS: FROM CONVENTIONAL ASSESSMENT TO PRECISION LIVESTOCK FARMING AND ARTIFICIAL INTELLIGENCE

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INTRODUCTION

The global poultry industry plays a central role in meeting the growing demand for high-quality animal protein owing to efficient feed utilization, rapid growth rates, and a relatively low environmental footprint compared with other livestock sectors. However, continued genetic selection for enhanced productivity and the intensification of commercial production systems have made flock management and animal-welfare assessment increasingly complex. In modern poultry houses accommodating thousands of birds simultaneously, long-term assessment of individual health status and behavior using conventional observation methods has become progressively more difficult (Neethirajan, 2025; Italiya et al., 2025).

Behavioral assessment in chickens has traditionally relied on direct human observation, scan sampling, focal-animal sampling, video recording, and ethogram-based analyses. Although these approaches have contributed substantially to our understanding of poultry behavior, their labor-intensive nature, observer dependence, and limited scalability constrain their use for continuous monitoring in large commercial flocks (Neethirajan, 2025; Bateson and Martin, 2021).

Recent advances in sensor technologies, computer vision, artificial intelligence (AI), and deep learning have expanded the possibilities for automated behavior monitoring in poultry production. These technologies enable continuous acquisition and automated analysis of behavioral and contextual data, while emerging developments such as multimodal AI, edge computing, digital twins, and explainable AI may further enhance the capabilities of next-generation monitoring systems (Taleb et al., 2025; Italiya et al., 2025).

This chapter reviews behavior monitoring in chickens from conventional observational methods to precision sensing and AI-based approaches, with emphasis on their applications, limitations, and future potential in precision poultry production.

2. Importance of Behavioral Monitoring in Chickens

Behavior is a non-invasive indicator that provides information on the physiological state, health, and welfare of chickens. Because behavioral changes may occur before measurable physiological or clinical signs become

apparent, monitoring behavior can facilitate the early detection of stress, disease, environmental disturbance, and management-related problems (Berckmans, 2017; Rowe et al., 2019).

Changes in locomotion, feeding and drinking, resting, feather pecking, aggression, panting, huddling, and vocalization may indicate impaired health or welfare. Conversely, natural behaviors such as dust bathing, perching, exploration, and other comfort behaviors provide information on the suitability of the housing environment and the extent to which birds can satisfy their behavioral needs (Colles et al., 2016; Li et al., 2020; Leishman and Riber, 2025).

Behavior is also associated with production performance. Feeding and drinking patterns, locomotor activity, nesting behavior, and resting time can provide useful information on feed efficiency, growth, egg production, reproductive performance, and responses to management practices (Rowe et al., 2019).

The importance of behavior monitoring has increased with the development of Precision Livestock Farming (PLF), which enables behavioral information to be collected and interpreted systematically over time. Integrating behavioral data with environmental, physiological, and production data may provide a more comprehensive understanding of animal responses and support data-driven management decisions (Li et al., 2020; Schillings et al., 2021; Zhang et al., 2022).

3.1 Ethogram-Based Behavioral Assessment

An ethogram is a standardized catalogue of observable behaviors defined using explicit criteria and is a fundamental tool in behavioral and welfare research. Standardized definitions improve the objectivity, repeatability, and comparability of behavioral observations across studies, observers, and production systems (Martin and Bateson, 2007; Leishman and Riber, 2025).

Chicken ethograms generally include feeding and drinking, locomotor, comfort, exploratory, resting, social, agonistic, and abnormal behaviors. Quantifying the frequency, duration, or proportion of observation time devoted to these behaviors enables behavioral patterns and time allocation to be compared across environments and production systems (Riber et al., 2018; Leishman and Riber, 2025).

Ethograms can be adapted to specific research objectives by emphasizing behavioral categories relevant to the experimental question, provided that standardized definitions and systematic recording procedures are maintained (Leishman and Riber, 2025).

Beyond conventional observation, ethograms also provide an essential basis for automated behavior recognition. Predefined behavioral labels can be used as ground-truth data for training and validating machine-learning models. Standardized ethograms therefore constitute a key reference framework for the development of automated behavior-monitoring systems (Okinda et al., 2020).

Table 1. Common behavioral categories included in chicken ethograms

Behavioral category	Behavior	Operational definition
Ingestive	Feeding	Pecking and consuming feed from the feeder
Ingestive	Drinking	Beak immersed in the drinker followed by swallowing
Locomotor	Walking	Forward movement using both legs
Resting	Sitting	Body supported by the sternum on the litter
Comfort	Preening	Grooming feathers using the beak
Comfort	Dust bathing	Scratching and rubbing the body in litter
Exploratory	Foraging	Pecking and scratching the litter without feed intake
Social	Allopreening	Grooming another bird
Agonistic	Aggressive pecking	Forceful peck directed toward another bird
Abnormal	Feather pecking	Pecking and pulling feathers from a conspecific

3.2. Locomotion Assessment

Locomotor ability is an important behavioral indicator of leg health and welfare in chickens. Impaired locomotion may restrict access to resources and the expression of normal behaviors and is commonly associated with leg disorders, lameness, and pain (Kestin et al., 1992). Gait scoring and the Latency-to-Lie (LTL) test are among the most commonly used conventional methods for assessing locomotor function.

3.2.1. Gait Score

Gait scoring is a subjective method used to assess walking ability and leg health in broiler chickens. In the conventional system developed by Kestin et al. (1992), birds are evaluated using a six-point scale ranging from 0 to 5 based on gait pattern, coordination, signs of lameness, and overall locomotor ability. During assessment, birds are encouraged to walk on a flat, unobstructed surface, and their locomotor characteristics are visually evaluated. A score of 0 indicates a normal, smooth, and well-coordinated gait with no detectable abnormalities; score 1 denotes a slight and difficult-to-define gait abnormality; score 2 represents a definite gait abnormality that does not substantially impair locomotion; score 3 indicates an obvious gait abnormality that affects the bird's ability to move; score 4 denotes severe locomotor impairment, in which the bird is capable of taking only a few steps; and score 5 indicates an inability to walk. Accordingly, increasing gait scores reflect progressively greater locomotor impairment and poorer leg health. The method is practical and requires minimal equipment; however, its reliance on observer-based assessment may affect repeatability and limit its suitability for continuous monitoring (Kestin et al., 1992; Webster et al., 2008).

3.2.2. Latency-to-Lie Test

The Latency-to-Lie (LTL) test indirectly assesses leg health by measuring how long a bird remains standing in shallow warm water before voluntarily sitting. Birds with impaired locomotor function generally sit sooner than birds with better walking ability (Weeks et al., 2002).

Associations between LTL responses and gait-based assessments support its use as a complementary measure of leg health in broilers. However, individual testing requirements and the time needed for assessment limit its

practicality in large commercial flocks (Weeks et al., 2002; Berg and Sanotra, 2003).

Both gait scoring and the LTL test provide useful reference measures of locomotor function but offer only periodic rather than continuous assessments. This limitation has encouraged the development of computer-vision and AI-based approaches for objective and continuous monitoring of locomotion and gait (Li et al., 2020).

3.3. Fear and Emotionality Tests

Fear and emotional reactivity in chickens are commonly assessed using standardized behavioral tests, including the Tonic Immobility Test (TI), Open Field Test (OFT), Novel Object Test (NOT), and Human Approach Test (HAT). Because these tests involve different stimuli and contexts, they assess complementary dimensions of fear related to restraint, social isolation, unfamiliar environments, novelty, and human presence (Jones, 1996; Forkman et al., 2007).

The TI test assesses fearfulness following brief physical restraint, with longer immobility generally interpreted as greater fearfulness. The OFT evaluates responses to social isolation and an unfamiliar environment using measures such as latency to move, locomotor activity, and vocalization. The NOT assesses responses to novelty through approach or avoidance of an unfamiliar object, whereas the HAT evaluates fear of humans based on birds' responses to human presence or approach (Jones, 1996; Forkman et al., 2007; Rasmussen et al., 2022).

Interpretation of these tests requires consideration of factors unrelated to fear itself. For example, locomotor impairment may affect approach or avoidance responses in broilers (Vasdal et al., 2018). Fear responses may also be stimulus-specific; early gentling has been reported to reduce fear toward humans without producing a comparable response to a novel object (Stocker et al., 2025). Thus, results from different fear tests should be interpreted as complementary rather than interchangeable measures of emotional reactivity.

These tests provide standardized measures of fear-related responses but may be influenced by handling, social isolation, locomotor ability, repeated testing, and testing conditions. Such factors should therefore be considered in experimental design and interpretation.

Table 2. Conventional tests used to evaluate fear-related behaviors in chickens

Test	Primary objective	Main variables measured	Interpretation	Key references
Tonic Immobility Test	Fearfulness	TI duration, induction attempts	Longer duration = higher fear	Jones (1996); Forkman et al. (2007)
Open Field Test	Exploration and anxiety	Latency to move, distance travelled, vocalizations	Lower activity = higher fear	Forkman et al. (2007); Campbell et al. (2019)
Novel Object Test	Response to novelty	Approach latency, pecking, interaction	Delayed approach = higher fear	Forkman et al. (2007); Rasmussen et al. (2022)
Human Approach Test	Fear of humans	Avoidance distance, birds approaching	Greater avoidance = higher fear	Welfare Quality® (2009); Rasmussen et al. (2022)

3.4. Social Behavior Assessment

Social behavior in chickens is conventionally assessed through direct observation or video recordings using standardized ethograms. Commonly evaluated behaviors include feather pecking, aggression, allopreening, social synchronization, and avoidance, which provide information on social interactions and group dynamics under different housing and management conditions (Estevez et al., 2007; Rodenburg et al., 2013).

Feather pecking and aggression are particularly relevant to welfare because of their association with plumage damage, injuries, and cannibalism. In contrast, affiliative behaviors such as allopreening and synchronized activities can provide information on positive social interactions and group cohesion. Social behavior can be quantified using measures such as the frequency, duration, or intensity of interactions, as well as social proximity, synchronization, and avoidance responses (Botreau et al., 2009; Carvalho et al., 2022; Rasmussen et al., 2022; Leishman and Riber, 2025).

Conventional assessment of social behavior is challenging because many interactions are brief, frequent, and dynamic, particularly in large or densely stocked flocks. Reliable identification of individuals and simultaneous

recording of multiple interactions may also be difficult, limiting comprehensive assessment through manual observation.

3.5. Feather, Plumage, and Footpad Scoring Systems

Feather, plumage, and footpad scoring systems are standardized animal-based methods widely used to assess the physical condition and welfare of chickens. These measures are incorporated into established welfare assessment frameworks, including the Welfare Quality® Assessment Protocol (Welfare Quality®, 2009; EFSA AHAW Panel, 2023).

3.5.1. Feather and Plumage Scoring

Feather and plumage scoring involves visual assessment of predefined body regions using standardized criteria for the extent and severity of feather loss and plumage damage. Poor feather condition may be associated with feather pecking, cannibalism, ectoparasite infestation, or suboptimal environmental and nutritional conditions. Standardized protocols such as Welfare Quality®, LayWel, and AssureWel provide defined scoring criteria and visual reference scales that improve consistency and repeatability among assessments (Tauson et al., 2005; Welfare Quality®, 2009; Decina et al., 2019).

3.5.2. Footpad Scoring

Footpad dermatitis (FPD) scoring assesses the presence and severity of footpad lesions, particularly in broiler chickens. Standardized visual scoring systems generally classify lesions from intact skin to severe ulceration or necrosis. Because FPD is strongly associated with litter and environmental conditions and severe lesions may cause pain and impair locomotion, it is widely used as an animal-based welfare indicator in commercial and scientific assessments (Berg, 1998; Haslam et al., 2006; EFSA AHAW Panel, 2023).

Although visual scoring systems are practical and relatively inexpensive, their reliability depends on standardized criteria and trained observers, and inter-observer variability may affect scoring consistency. Nevertheless, they remain important reference methods for welfare assessment.

Table 3. Conventional scoring systems used to assess feather, plumage, and footpad condition in chickens

Scoring system	Assessment method	Main variables evaluated	Welfare significance	Representative references
Feather/Plumage Scoring	Visual assessment of predefined body regions	Feather loss, feather damage, plumage integrity	Feather pecking, cannibalism, long-term welfare	Tauson et al. (2005); Welfare Quality® (2009); Decina et al. (2019)
Footpad Dermatitis (FPD) Scoring	Visual lesion scoring	Lesion severity, ulceration, necrosis	Litter quality, pain, locomotor impairment	Berg (1998); Haslam et al. (2006); EFSA AHAW Panel, 2023

3.6. Time Budget and Activity Assessment

Time budget analysis quantifies the proportion of an observation period allocated to different behavioral activities, providing a temporal representation of behavioral organization. It enables comparisons of activity patterns across experimental treatments, housing systems, environmental conditions, and management practices (Lehner, 1996; Colwill and Suchak, 2022; EFSA AHAW Panel, 2023; Leishman and Riber, 2025).

Time budgets are typically derived from direct observations or video recordings using predefined behavioral categories and systematic sampling procedures. Behaviors may be recorded continuously or at predetermined intervals, with observations expressed as durations or proportions of the total observation period (Bateson and Martin, 2021).

The reliability of time budget estimates depends on the sampling design, including observation duration, sampling interval, time of day, and distribution of observation periods. Restricted sampling may fail to capture short-term or diurnal variation, whereas prolonged video analysis can be labor-intensive and time-consuming, particularly when large numbers of birds or behavioral categories are evaluated (Lehner, 1996; Bateson and Martin, 2021).

3.7. Integrated Welfare Assessment Protocols

Integrated welfare assessment protocols combine animal-based, resource-based, and management-based indicators to provide a

multidimensional evaluation of poultry welfare. Among the established approaches are the Welfare Quality® Assessment Protocol and the Transect Walk method, which differ in their scope, sampling strategy, and practical application under commercial conditions (Welfare Quality®, 2009; Marchewka et al., 2013).

The Welfare Quality® Assessment Protocol is structured around four principles: Good Feeding, Good Housing, Good Health, and Appropriate Behavior. These principles integrate multiple welfare indicators within a standardized framework for flock- or farm-level assessment. Although comprehensive, implementation can be time-consuming and may require handling and individual assessment of sampled birds, limiting its practicality in large commercial flocks (Welfare Quality®, 2009; Botreau et al., 2009; de Jong et al., 2012; Marchewka et al., 2013).

The Transect Walk method provides a more practical approach for flock-level welfare assessment by allowing trained observers to record predefined welfare indicators while walking along predetermined transects within the poultry house. By avoiding capture and individual examination of sampled birds, the method enables assessment of a larger proportion of the flock with minimal handling and disturbance (Marchewka et al., 2013).The principal integrated welfare assessment approaches used in poultry production are summarized in Table 4.

Table 4. Major integrated welfare assessment approaches used in poultry production

Protocol	Primary focus	Main welfare indicators	Advantages
Welfare Quality®	Comprehensive welfare assessment	Feeding, housing, health, behavior, gait score, footpad dermatitis, feather condition, fear of humans	Comprehensive and scientifically validated
AssureWel	Practical welfare auditing	Feather loss, footpad dermatitis, lameness, mortality, cleanliness	Rapid and suitable for commercial farms
Transect Walk Method	Whole-flock welfare assessment	Lameness, sick birds, immobility, feather loss, wounds, dirty plumage, mortality	Fast, non-invasive, minimal bird handling

4. Precision Behavior Monitoring Technologies

Precision behavior monitoring technologies enable systematic collection of behavioral and contextual data at individual or flock level under poultry production conditions. Available sensing approaches differ in their operating principles, spatial and temporal resolution, behavioral specificity, and capacity for individual identification (Berckmans, 2017; Li et al., 2020).

The suitability of a monitoring technology depends on the target behavior, housing system, flock size, monitoring duration, and intended application. Therefore, technology selection should be based on the biological objective and practical requirements of the monitoring system rather than on technological capability alone (Ben Sassi et al., 2016; Berckmans, 2017).

Table 5. Principal precision technologies used for behavioral monitoring in chickens

Technology	Main data source	Typical applications	Major advantages	Major limitations
Camera-based systems	RGB, depth, thermal images/video	Locomotion, activity, feeding, social behavior, flock distribution	Non-invasive; continuous; high-throughput	Occlusion; lighting; computational requirements
Wearable sensors	Accelerometers, IMUs	Activity, posture, locomotion	Detailed individual-level measurements	Attachment, battery life, weight, limited flock coverage
Acoustic monitoring	Audio signals	Vocalizations, distress, flock activity	Non-invasive; independent of lighting	Background noise; limited individual identification
Environmental sensors	Temperature, humidity, gases, light	Environmental context of behavioral responses	Continuous environmental monitoring	Indirect measure of behavior

4.1. Camera-Based Monitoring Systems

Camera-based systems are widely used in precision poultry monitoring because they enable non-contact assessment of bird activity, movement, posture, and spatial distribution at flock or individual level (Fernández et al., 2018).

RGB cameras capture visible-light images and are widely used because of their relatively low cost and compatibility with automated image analysis. They can support monitoring of locomotion, activity, resource use, and spatial distribution (Li et al., 2020; Dawkins et al., 2021). RGB-D cameras additionally provide depth information, enabling three-dimensional characterization of posture, movement, and spatial relationships when two-dimensional images are insufficient (Aydin, 2017; Zhang et al., 2022).

Thermal cameras detect infrared radiation emitted from the body surface and provide contact-free information on surface temperature and thermal distribution. In poultry, thermal imaging can complement behavioral monitoring by detecting responses associated with thermal conditions and heat exposure (Naas et al., 2014). Multi-camera systems combine synchronized views to improve spatial coverage and reduce occlusion, particularly for individual tracking and analysis of interactions among birds (Xiao et al., 2023; Nidhi et al., 2025).

Camera-based monitoring remains sensitive to high stocking density, occlusion, variable illumination, complex backgrounds, and changes in bird size and appearance during growth. These factors may reduce image quality and the reliability of behavioral measurements. Computational approaches for addressing these challenges, including detection, segmentation, pose estimation, and tracking, are discussed in Section 5. The principal camera technologies used for behavioral monitoring in chickens are summarized in Table 6.

Table 6. Camera-based technologies used for behavioral monitoring in chickens

Camera technology	Data type	Main applications	Major advantages	Major limitations
RGB camera	Visible-light images/video	Activity, locomotion, feeding, spatial distribution	Low cost; widely available; suitable for automated image analysis	Sensitive to illumination; occlusion in dense flocks
RGB-D (Depth) camera	Color + depth	Posture, gait, locomotion, spatial relationships	Three-dimensional information; improved spatial characterization	Increased data and processing requirements
Thermal camera	Infrared/thermal images	Thermal responses, surface temperature, activity distribution	Non-contact thermal assessment; no visible-light requirement	Limited behavioral detail; primarily complementary to behavioral monitoring
Multi-camera system	Synchronized multi-view images/video	Individual tracking, social interactions, spatial behavior	Greater spatial coverage; reduced occlusion	Increased system and computational complexity

4.2. Wearable Sensor Technologies

Wearable sensor technologies enable individual-level monitoring by attaching sensing or identification devices directly to birds. Depending on the technology, these devices can provide information on activity, posture, location, and resource use over extended monitoring periods (Berckmans, 2017; Li et al., 2020).

Accelerometers measure linear acceleration along one or more axes and can be used to characterize activity and movement patterns. When combined with appropriate classification algorithms, acceleration data can distinguish behavioral states such as walking, resting, and feeding. Their performance is influenced by sensor placement, sampling frequency, attachment method, and the behaviors being classified (Li et al., 2020; Yang et al., 2021).

Radio-frequency identification (RFID) systems enable automated identification of individual birds using electronic tags detected by antennas positioned at predefined locations. RFID has been used to quantify resource use, nest-box and range use, and movement between functional areas, providing individual-level information on spatial and resource-use patterns (Oliveira et al., 2019; Gebhardt-Henrich et al., 2023; Alindekon et al., 2023).

Inertial measurement units (IMUs) combine accelerometers with gyroscopes to measure linear acceleration and angular velocity. The additional motion information can improve characterization of posture changes and more complex movement patterns compared with accelerometers alone. However, data quality depends on sensor placement, device configuration, sampling frequency, and data-processing methods.

Despite their capacity for continuous individual-level monitoring, wearable technologies face practical limitations related to device attachment, weight, battery life, durability, data transmission, and cost. Their application to large commercial flocks is further constrained by the need to instrument individual birds and by the limited representativeness of measurements obtained from only a subset of the flock (Khan et al. 2025). The principal wearable sensor technologies used for behavioral monitoring in chickens are summarized in Table 7.

Table 7. Comparison of wearable sensor technologies used for behavioral monitoring in chickens

Technology	Monitoring principle	Typical behavioral applications	Major advantages	Major limitations
Accelerometers	Measure linear acceleration along one or more axes	Walking, standing, resting, feeding, locomotion, activity level	Continuous individual monitoring; lightweight; suitable for long-term activity assessment	Limited behavioral specificity without advanced algorithms; battery life constraints
RFID	Identification of tagged birds using radio-frequency signals detected by antennas	Feeding and drinking visits, nest box use, range use, movement between functional areas	Accurate individual identification; long-term monitoring; automated resource-use recording	Requires fixed antennas; detects birds only within antenna range; infrastructure cost
Inertial Measurement Units (IMUs)	Combine accelerometers and gyroscopes to measure linear acceleration and angular velocity	Posture, locomotion, wing flapping, grooming, perching, behavioral transitions	High-resolution movement analysis; improved recognition of complex behaviors	Higher cost; increased power consumption; more complex data processing

4.3. Acoustic Monitoring Systems

Acoustic monitoring systems use microphones to capture vocal and non-vocal sounds generated within poultry houses. Unlike camera-based systems, acoustic sensing does not depend on lighting conditions and is less affected by visual occlusion, making it suitable for continuous flock-level monitoring (Coutant et al., 2024).

Chicken vocalizations contain biologically relevant information associated with behavioral states and responses to environmental conditions. Acoustic features such as call frequency, intensity, duration, and temporal structure may vary with feeding-related activity, social communication, distress, and thermal conditions. Non-vocal sounds, including coughing and

sneezing, may also provide information relevant to respiratory health (Lee et al., 2015; Mahdavian et al., 2021; Coutant et al., 2024).

The reliability of acoustic monitoring depends on microphone placement, recording quality, and the acoustic characteristics of the poultry house. Background noise from ventilation, feeding equipment, bird movement, and overlapping vocalizations can interfere with target signals, making noise reduction, sound-source separation, and reliable event identification important technical challenges. Automated approaches for acoustic feature extraction and classification are discussed in Section 5.

4.4. Environmental Sensor Technologies

Environmental sensor technologies provide contextual information for behavioral monitoring by continuously measuring conditions within poultry houses. Unlike animal-based sensing technologies, they do not directly quantify behavior but characterize environmental factors that may influence behavioral responses. Commonly monitored variables include air temperature, relative humidity, ammonia (NH_3) and carbon dioxide (CO_2) concentrations, light intensity, and air velocity (Berckmans, 2017; Li et al., 2020).

Temperature and relative humidity sensors characterize thermal conditions, while ammonia and carbon dioxide sensors provide information on air quality and ventilation. Light and air-velocity sensors characterize illumination and airflow conditions, respectively. These measurements can support interpretation of temporal and spatial variation in behavioral patterns in relation to the surrounding environment (Rowe et al., 2019; Li et al., 2020).

The reliability of environmental measurements depends on their spatial and temporal representativeness. Conditions within poultry houses may vary with sensor location, ventilation patterns, stocking density, litter condition, and proximity to heating or cooling systems. Consequently, sensor placement, calibration, sampling frequency, maintenance, and spatial coverage should be considered when environmental data are integrated with behavioral observations (EFSA AHAW Panel, 2023).

4.5. Multi-Sensor Integrated Monitoring Systems

Multi-sensor monitoring systems combine complementary data from two or more sensing modalities to provide a more comprehensive representation of chicken behavior and its environmental context. Depending on the monitoring

objective, camera data may be integrated with environmental sensors, while RFID, wearable, or acoustic sensors can provide additional information on individual identity, movement, resource use, or vocal activity. Such integration can compensate for modality-specific limitations and provide information that cannot be obtained from a single data source alone (Berckmans, 2017; Li et al., 2020; Coutant et al., 2024).

A central component of multi-sensor monitoring is sensor fusion, in which data from different sensing modalities are synchronized and combined. Fusion may occur at the raw-data, feature, or decision level, depending on the system architecture. Its effectiveness depends on temporal synchronization, compatibility among data formats and sampling rates, sensor calibration, and the reliability of individual data sources (Essien and Neethirajan, 2025).

Despite their potential, multi-sensor systems introduce additional requirements for data transmission, storage, processing, synchronization, and interoperability. Increasing numbers of sensors and larger multimodal datasets may further increase computational demands and system complexity, potentially limiting scalability under commercial conditions. Robust analytical methods and standardized integration frameworks are therefore important for translating multimodal data into biologically meaningful information.

5. Artificial Intelligence Applications in Chicken Behavior Monitoring

Artificial intelligence (AI) provides the analytical framework for transforming data generated by precision monitoring technologies into interpretable behavioral information. AI-based approaches can detect, classify, quantify, and track behavioral patterns that would otherwise require extensive manual analysis (Li et al., 2020).

Within this framework, machine learning (ML) algorithms learn patterns from predefined features, whereas deep learning (DL) models can automatically learn hierarchical representations from complex data such as images, video, audio, and sensor signals (LeCun et al., 2015; Goodfellow et al., 2016). In camera-based applications, computer vision combined with deep learning has substantially advanced bird detection, classification, tracking, and behavior recognition (Li et al., 2020; Guo et al., 2022).

AI applications in chicken behavior monitoring include automated activity recognition, locomotion assessment, social interaction analysis, individual identification and tracking, and multimodal data interpretation. However, system performance depends on data quality and representativeness, annotation accuracy, environmental variability, model generalizability, and computational requirements (Guo et al., 2022; Yang et al., 2023).

Table 8. Major AI applications and analytical approaches for chicken behavior monitoring

AI application	Typical data source	Common analytical approaches	Main behavioral outputs
Activity recognition	RGB/depth video, wearable sensors	CNNs, object detection, classification	Feeding, drinking, resting, walking, comfort behaviors
Locomotion and gait assessment	RGB/depth video	CNNs, pose estimation, tracking	Gait, posture, locomotor activity, lameness-related features
Social behavior analysis	Video	Object detection, tracking, spatiotemporal analysis	Feather pecking, aggression, social interactions
Individual identification and tracking	Video, RFID	Detection, re-identification, multi-object tracking	Identity, trajectories, resource use
Multimodal analysis	Video, audio, wearable and environmental data	Multimodal learning, sensor fusion	Integrated behavioral and environmental interpretation

5.1. Automated Activity Recognition

Automated activity recognition uses AI-based methods to identify and classify behavioral states or events from data generated by precision monitoring technologies. In poultry, these approaches can quantify the occurrence, frequency, and duration of behaviors such as feeding, drinking, walking, resting, and comfort activities (Fernandez et al., 2018).

Camera-based activity recognition commonly involves detecting birds within images or video and subsequently classifying their behavior. Deep learning frameworks, including convolutional neural networks (CNNs) and

object-detection models such as You Only Look Once (YOLO), have improved the automated recognition of routine chicken behaviors under experimental and production conditions (Redmon et al., 2016; Guo et al., 2022).

The performance of automated activity recognition depends on image quality, bird density, occlusion, behavioral similarity, and the quality and representativeness of annotated training data. Behaviors with similar postures or short durations may be particularly difficult to distinguish, while models developed under specific experimental conditions may not generalize reliably across different breeds, ages, housing systems, or camera configurations. Robust validation across diverse production conditions is therefore essential before these systems can be applied reliably at commercial scale (Li et al., 2020; Guo et al., 2022; Yang et al., 2023).

5.2. AI-Based Locomotion and Gait Assessment

Artificial intelligence and computer vision enable automated quantification of locomotor and gait-related characteristics from image and video data. Unlike observer-based gait scoring, these approaches extract objective movement features that can be used to characterize locomotor patterns and detect deviations associated with impaired walking ability (Li et al., 2020).

AI-based locomotion assessment can quantify features such as walking speed, movement trajectories, activity level, body posture, and spatial displacement over time. Depth-sensing technologies, including RGB-D imaging, additionally provide three-dimensional information that can improve the characterization of posture and movement-related features (Aydin, 2017; Fodor et al., 2025).

More advanced approaches combine pose estimation and tracking to characterize locomotion from the positions of anatomical landmarks and the trajectories of individual birds across consecutive frames. These methods may enable detection of subtle locomotor changes that are difficult to capture using categorical gait scores alone, but their performance depends on reliable detection and tracking under variable production conditions.

The reliability of AI-based locomotion assessment is influenced by image quality, annotation accuracy, and variation in bird age, size, and locomotor characteristics. Standardized datasets and validation across diverse

production conditions are therefore needed to support robust application under commercial poultry conditions.

5.3. Automated Social Behavior Analysis

Artificial intelligence and computer vision enable automated detection and quantification of social interactions from poultry video data. These approaches use spatial and temporal relationships among birds to identify interaction events and derive quantitative measures of social behavior (Subedi et al., 2023).

Feather pecking is an important target for automated social behavior analysis because interactions are often brief and must be distinguished from visually similar pecking-related activities. Subedi et al. (2023) applied a YOLOv5-based deep learning approach to detect feather-pecking behavior in laying hens, demonstrating the feasibility of object detection for automated identification of these events.

Automated recognition of aggressive interactions generally requires both detection and temporal information. Object detection and tracking can be combined with spatiotemporal features to characterize interactions between birds across consecutive video frames. However, the short duration and rapid movement of aggressive events can complicate their differentiation from other social interactions (Xue et al., 2025).

Beyond specific interaction events, tracking data can be used to characterize broader social and collective patterns. Individual trajectories can provide measures of inter-individual distance, spatial proximity, movement synchronization, clustering, and flock distribution, enabling quantitative assessment of social organization and collective dynamics (Neethirajan, 2022; Essien and Neethirajan, 2025).

5.4. AI-Based Individual Identification and Tracking

Individual identification and tracking aim to maintain a consistent identity for each bird across image or video sequences, allowing behavioral information to be linked to specific individuals over time. This enables the development of longitudinal behavioral profiles and the quantification of individual variation that may not be apparent from flock-level measurements (Li et al., 2020; Neethirajan, 2022).

Individual identification can be achieved using marker-based or markerless approaches. Marker-based systems use physical identifiers, such as leg bands or RFID tags, whereas markerless approaches distinguish birds using visual features and spatiotemporal information without requiring physical identifiers (Oliveira et al., 2019; Alindekon et al., 2023; Neethirajan, 2022).

AI-based tracking typically involves bird detection, association of detections across consecutive frames, and maintenance of identity over time. Multi-object tracking (MOT) enables simultaneous tracking of multiple birds and generation of individual trajectories, while re-identification (Re-ID) methods can recover identities after temporary loss caused by occlusion or movement outside the camera field of view. Multi-view and multi-camera approaches may further support identity continuity across different viewpoints (Xiao et al., 2023; Nidhi et al., 2025).

Long-term markerless tracking remains challenging because chickens may have similar visual appearances, while age, posture, viewpoint, occlusion, and changing production conditions can alter discriminative features. Recent studies have explored deep feature representations and transformer-based architectures for chicken identification and Re-ID, but their performance depends on representative datasets that capture such variability (Kern et al., 2025; Lamping et al., 2025).

Table 9. Representative approaches for individual identification and tracking in chickens

Approach	Main objective	Data/identifier	Major advantage	Major limitation
Marker-based identification	Individual identification	RFID, leg bands	Reliable identity assignment	Requires physical markers
Markerless visual identification	Individual recognition	RGB images/video	No physical markers required	Similar appearance among birds
Multi-object tracking (MOT)	Maintain identities across frames	Video sequences	Individual trajectories	Occlusion and identity switches
Re-identification (Re-ID)	Recover/match identity across observations	Visual features	Longer-term identity preservation	Appearance and viewpoint variation

6. Future Perspectives

Future developments in poultry behavior monitoring are expected to move beyond the automated recognition of predefined behaviors toward systems capable of integrating heterogeneous information, interpreting complex behavioral dynamics, and generating actionable information for precision management. Achieving this transition will require greater emphasis on model generalizability, biological validation, interoperability, and reliable performance under diverse commercial production conditions.

Multimodal AI and data fusion will be important components of this transition. Future research should focus not simply on combining additional data sources but on identifying combinations of visual, acoustic, wearable, environmental, and production data that provide biologically meaningful improvements in behavioral interpretation. Robust fusion strategies will also be needed to accommodate heterogeneous data collected at different temporal and spatial scales and to maintain performance when individual data streams are incomplete or degraded (Li et al., 2020; Neethirajan, 2022).

Foundation models and vision-language models (VLMs) represent an emerging research direction with potential applications in poultry behavior monitoring. Compared with models developed for narrowly defined tasks and predefined behavioral classes, more generalizable models may facilitate knowledge transfer across monitoring tasks and production environments and reduce dependence on extensively annotated task-specific datasets. However, these potential advantages require rigorous validation using representative poultry datasets rather than extrapolation from other livestock species or general computer-vision applications (Ahmad et al., 2025).

Edge AI and explainable artificial intelligence (XAI) address complementary requirements for practical deployment. Edge AI enables model inference close to the point of data acquisition and may reduce communication latency, bandwidth requirements, and dependence on continuous transmission of high-volume data (Cakic et al., 2023; Tong et al., 2024). XAI may improve transparency by identifying behavioral, spatial, acoustic, or environmental features contributing to model predictions, which is particularly important when automated outputs support biological interpretation or management decisions (Hoxhallari et al., 2022). Progress in both areas should be

accompanied by standardized datasets, annotation protocols, benchmarking procedures, and external validation.

Digital twins represent a longer-term opportunity for integrating behavioral, environmental, physiological, and production information into dynamically updated representations of individual birds, subgroups, or flocks. Such systems could support modeling of behavior–environment interactions, prediction of future states, and evaluation of alternative management scenarios. However, livestock digital twins remain at an early stage and require reliable longitudinal data, validated computational models, interoperable sensing platforms, and biologically meaningful links between digital representations and real animals (Symeonaki et al., 2024; Arulmozhi et al., 2024).

7. CONCLUSIONS

Behavioral monitoring is fundamental to assessing chicken welfare, health, and responses to production conditions. Conventional assessment methods remain essential for defining behavioral outcomes and validating emerging monitoring technologies, but their limitations in continuous and large-scale observation have increased the need for more objective and automated approaches.

Precision sensing technologies, combined with artificial intelligence, have substantially expanded the capacity to detect, classify, quantify, and track behavior at individual and flock levels. Their value extends beyond automating conventional observations by enabling behavioral patterns to be evaluated at temporal and spatial scales that are difficult to achieve through manual assessment alone.

The transition from experimental systems to reliable commercial applications requires further progress in model robustness and generalizability, data standardization, individual identity preservation, multimodal integration, and biological validation. Ultimately, the value of these technologies will depend not only on algorithmic performance but on their ability to generate biologically meaningful and actionable information that supports poultry welfare, health management, and production efficiency.

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CHAPTER 5

DETERMINING THE EFFECTS OF DIFFERENT SOWING DENSITIES AND SOWING TIMES ON SOME YIELD COMPONENTS AND GRAIN YIELD IN DRIED BEANS*

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INTRODUCTION

The importance of all edible grain legumes, such as beans, in the human diet is an indisputable fact. Furthermore, these plants' ability to fix atmospheric nitrogen is also significant in terms of leaving behind clean and fertile soil for subsequent crops. Bean cultivation is becoming increasingly important in today's world, where environmentalism and sustainable agricultural practices are becoming more widespread (Şehirali, 1988) It is reported that 22 per cent of plant-based proteins and 7 per cent of carbohydrates in the global human diet, as well as 38 per cent of proteins and 5 per cent of carbohydrates in animal feed, are derived from food legumes (Adak et al., 2010). Dried beans (*Phaseolus vulgaris* L.) are the most widely consumed of the edible grain legumes (Sat, 1997). Depending on the variety and growing conditions, the protein content of dried beans varies between 17 per cent and 35 per cent (average 22 per cent). Furthermore, they are an extremely rich source of phosphorus, iron, vitamin B1 and dietary fibre (Robinson, 1987). As dried beans are an inexpensive source of protein, they are produced in many countries to meet the nutritional needs of the population, particularly in developing nations; to support the livelihoods of rural populations; due to their place in the traditional culinary cultures of Latin American countries; and because they are traded internationally.

Although dry beans rank first in the world among food legumes, with a cultivation area of 34,495,662 ha and a production of 30,434,280 tonnes, in our country they rank third after chickpeas and lentils, with a cultivation area of 84,786 ha and a production of 220,000 tonnes. Whilst the average yield per decare in countries growing dried beans worldwide is 88 kg, in our country this figure is around 259 kg (Anonymous, 2018). Of the 840,786 da of cultivated area in our country, 68,723 decares are cultivated in Nevşehir province. A yield of 265 kg per decare was achieved. The district of Derinkuyu, meanwhile, has a cultivation area of 45,000 dekar (Anonymous, 2017). When examining dried bean production in our country, the provinces with the largest cultivated areas are Konya (14,811 ha), Karaman (10,151 ha), Erzincan (2,570 ha), Niğde (10,348 ha) Nevşehir (6,872 ha), Samsun (2,343 ha) and Kahramanmaraş (2,287 ha)). These seven provinces, which account for the vast majority of the country's bean cultivation area, demonstrate that bean production is concentrated in specific regions. This concentration of bean cultivation in the

country carries the risk that, in any given year, should weather conditions be unfavourable or there be a high incidence of diseases and pests in these provinces where the most beans are grown, there could be a significant decline in bean yield and, consequently, in the country's total bean production. To mitigate this risk, research into yield, adaptation and variety development must be carried out for each region, with varieties that stand out for their superior characteristics and

In order to address this shortcoming, it is of great importance to carry out research into yield, adaptation and variety development for each region, to make varieties and candidate lines that stand out for their superior characteristics available to growers, and thereby to expand bean production across the country.

In Nevşehir, a major centre for bean production in Turkey, an examination of the bean production system revealed that, in addition to seed selection, there were incorrect practices and issues regarding sowing time and sowing density. For these reasons, the aim of this study was to determine the effect of different sowing dates and plant densities on the grain yield and certain grain yield characteristics of dry beans.

MATERIALS AND METHODS

Material

The study was conducted in crop production areas under farming conditions in the Derinkuyu district of Nevşehir. The trial site is situated 28.5 km from Nevşehir, at an altitude of 1,305 m, with a latitude of 38°24'25.1 North and a longitude of 34°44'06.2' East. "

The Alberta bean variety, which is widely cultivated in the Derinkuyu region of Nevşehir, was used in the trial.

According to the soil analysis results, the soil is found to be poor in organic matter. However, it is found to be rich in potassium, phosphorus and calcium. The trial site has a slightly alkaline, clay-loam soil structure.

Table 1. Physical and chemical properties of the experimental site.

Specifications	0-30 cm Soil depth
pH	8.0
Total Salt %	0.012
Organic Matter %	0.35
Phosphorus ((P2O5)	2.17
Potassium (K2O (kg/da))	101.2
Lime % (CaCO3)	16.3
Saturation %	34
Nitrogen %	0.098

Although Nevşehir province is situated in the Central Anatolia climate zone, it experiences hot, dry summers and cold, wet winters. Data regarding monthly total precipitation, relative humidity, and average monthly temperatures for the trial site were obtained from the General Directorate of Meteorology and are presented in Table 2.

The trial crop was sown in April 2018 and harvested in August 2018. Data recorded in 2018 by the station operated by the General Directorate of Meteorology (under the Ministry of Agriculture and Forestry) in Nevşehir were requested and analyzed.

Table 2. Climate data for Kırşehir Province, where the experiment was conducted, for 2018 and the long-term average

	Average Monthly		Total Monthly		Relative Humidity	
	long-term	2018	long-term	2018	long-term	2018
January	-0.3	1.07	43.3	72.3	78.6	81.65
February	1.0	6.55	40.9	12.8	74.6	68.58
March	5.0	10.04	47.0	56.4	67.6	66.17
April	10.1	13.17	49.5	12.8	63.6	49.14
May	14.4	16.32	57.8	47.5	59.9	64.80
June	18.2	20.28	34.1	46	53.5	53.37
July	21.3	23.5	8.3	1.2	47.3	43.4
August	21.2	22.6	4.5	3.6	45.3	39.16
September	17.3	18.88	12.1	0.9	51.8	45.65
October	12.1	13.39	30.4	57.4	62.4	62.27
November	6.4	7.07	35.1	8	71.5	66.8
December	1.8	2.5	49.8	103.4	77.8	81.35
Total			412.8	422.3		
Average	10.70	12.94			62.78	60.134

*Source: https://www.mgm.gov.tr/

An examination of the temperature data in Table 2 reveals that the average temperatures in 2018 were higher than the long-term averages. It is observed that the highest temperature in 2018 occurred in July at 23.5°C, which was 2.2°C above the long-term average.

An examination of precipitation data reveals that rainfall levels in April and May 2018 were lower than the long-term average, whereas the rainfall in June 2018 exceeded the long-term average.

Method

The study was conducted in triplicate according to a split-plot design with randomised blocks. In the study, five different sowing dates (20 April. 4 May, 18 May, 1 June and 15 June) were assigned to the main plots, whilst five different sowing densities (40x8, 40x10, 40x12, 40x14 and 40x16) were assigned to the sub-plots. In the study, the row spacing was set at a fixed 40 cm, whilst the in-row spacing was set at five different levels (8, 10, 12, 14 and 16 cm). The seed rates to be applied according to the different planting densities were as follows: 40x8 cm – 11.2 kg/da seed, 40 × 10 cm: 9.0 kg/da of seed, 40 × 12 cm: 7.5 kg/da of seed, 40 × 14 cm: 6.4 kg/da of seed, and 40 × 16 cm: 5.6 kg/da.

The plots were laid out with row lengths of 5 m. Each plot consisted of 5 rows of plants. The distance left between plots was 40 cm. A spacing of 2.5 metres was maintained between blocks. The trial comprised a total of 75 plots, with 25 plots in each block, depending on the factor combinations. Initial soil preparation at the trial site was carried out in October 2017. Soil preparation was completed by ploughing prior to sowing.

The trial was established on an irrigable field and set up in accordance with the most suitable maintenance and cultivation techniques for beans in the region. Based on the soil analysis results obtained from the trial, 15 kg/da of DAP (18-46-0) fertiliser was applied by hand at the time of sowing. Irrigation was carried out whenever sufficient rainfall was not received at the time of sowing, until the soil reached field capacity. Subsequently, after the necessary checks had been carried out, all plots were irrigated using the drip irrigation method. Weed control was carried out by hoeing and hand-weeding as and when required.

The harvest of the plants, which were frequently monitored in the trial area, was carried out using a sickle. After leaving a 0.5 m buffer zone from the plot heads and one row on each side to account for edge effects, the remaining plants within the plot area (the two central rows) were harvested by cutting with a sickle. The plots harvested with the sickle were placed into pre-prepared, labelled bags and prepared for threshing. Subsequently, the necessary measurements were taken and the crops were threshed in the laboratory.

Characteristics Examined in the Study: The characteristics examined for each plot were determined on sample plants selected at random from the plants in the middle row of each plot, as set out below.

Number of plants per square metre (plants/m²): This will be determined by counting the plants within one square metre once emergence is complete.

Plant height (cm): In each plot that has reached harvest maturity, the distance from the soil surface to the tip of the plant will be measured on 10 randomly selected plants, and the average will be recorded in 'cm'

Initial pod height (cm): In each plot that has reached harvest maturity, the distance in 'cm' between the soil surface and the point where the pod attached to the fruit stalk is located on the plant—measured on the 10 plants selected at random—shall be recorded.

Number of main stems (number/plant): In each plot that has reached harvest maturity, the number of main stems will be counted on 10 plants selected at random, and the average will be recorded as "number/plant".

Grain yield (kg/da): After excluding the edge effect in each plot, the plants in the remaining area will be allowed to dry naturally following harvest, threshed, and their grain weights measured and recorded in "kg/da".

Hundred-grain weight (g): From the grains obtained from each plot, 100 grains will be weighed in quadruplicate, and the result will be recorded in 'g'.

Statistical analysis: Evaluation of Data Obtained in the Study: The data obtained were analysed using the MSTAT-C software package to perform analysis of variance (ANOVA) according to the 'Plots Split into Randomised Blocks' experimental design. Means were grouped using the 'Duncan's test'

RESULTS AND DISCUSSION

This study was conducted to determine the effects of different planting densities and planting times on grain yield and certain yield components in the Alberta bean variety. Characteristics such as the number of plants per m² (number), plant height (cm), height of the first pod (cm), number of main branches (number), grain yield (kg/da), hundred-seed weight (g) were examined.

Number of Plants: When the study was analysed in terms of the number of plants per square metre, it was found that different planting densities and planting times had a statistically significant effect on the number of bean plants per square metre. The same study also revealed that the interaction between planting time and planting density had a highly significant effect (Table 3). It has been established that the sowing date has a significant effect on the number of plants per square metre in beans. An examination of Table 4 reveals that the highest number of plants per square metre 19.73 plants was obtained from sowings carried out on 18 May. It was found that the regular rainfall during these periods ensured sufficient soil moisture for germination, facilitating the complete germination of the seeds used and thereby having a positive effect on the number of plants per square metre. It was found that plantings carried out on 20 April and 4 May yielded 18.53 and 17.40 plants per square metre, respectively. As sowing was delayed, the number of plants per square metre decreased; due to a reduction in soil moisture during a period of high temperatures, only 6.13 plants per square metre were recorded on 15 June. The beans sown on 15 June were exposed to high-temperature stress, resulting in wilting and scorching of the plants. Consequently, this led to a reduction in the number of plants per square metre, with the lowest number of plants per square metre being recorded in the sowings carried out on that date. Similarly, from a statistical perspective, it was found that the lowest number of plants per square metre 5.86 was recorded in the sowings carried out on 1 June.

Table 3: Results of the analysis of variance for the number of plants per square metre under different planting densities and planting times

	S D	Sum of	Mean of	F value	Significance
Repetition	2	15.70	7.85	2.70	0.1267
Sowing Time (ST)	4	2878.93	719.73	247.89	0.0000**
Error 1	8	23.22	2.90		
Sowing Density (SD)	4	804.66	201.16	290.14	0.0000**
ST × SD Int.	16	214.40	13.40	19.32	0.0000**
Error 2	40	27.73	0.69		
General	74	3964.66			

CV (%): 6.15;

* Significant at the P ≤ 0.05 level, ** Significant at the P ≤ 0.01 level, Ns Not significant

Table 4: Means for the number of plants per square metre under different sowing densities and sowing times, and the results of the Duncan test for comparisons between means

Sowing Times	Planting Density					
	8 cm	10 cm	12 cm	14 cm	16cm	Avg.
20 April	25.00 b	21.00 d	15.66 fgh	13.00 ıj	12.33 j	17.40 b
4 May	25.67 b	20.33 d	17.00 ef	15.33 gh	14.33 hı	18.53 ab
18 May	27.33 a	23.33 c	17.67 e	16.00 fg	14.33 hı	19.73 a
1 June	7.33 kl	6.33 lm	6.00 lmn	5.33 mno	4.33 o	5.86 c
15 June	8.66 k	7.33 kl	5.66 mno	4.66 no	4.33 o	6.13 c
Avg.	18.80 a	15.66 b	12.40 c	10.86 d	9,93 e	

*The difference between means denoted by the same letter is not significant (Duncan's test, p ≤ 0.05)

The effect of sowing density on the number of plants per square metre in beans was found to be as significant as that of sowing time. The highest number of plants per square metre was observed in sowings carried out in 8 cm rows, where 31.3 seeds were sown; it was found that 18.80 plants were obtained from these seeds. As the row spacing increased, the number of seeds used decreased, and consequently, the number of plants per square metre decreased in direct proportion. It was found that sowing at 12 cm between rows yielded 15.66 plants, sowing at 14 cm between rows yielded 12.40 plants, and sowing at 14 cm between rows yielded 10.86 plants. The lowest number of plants per square metre was obtained from sowing at 16 cm row spacing; it was found that 15.6 seeds were sown for 16 cm row spacing, yielding 9.93 plants.The study found

that the interaction between sowing date and sowing density had a significant effect on the number of plants per square metre. It was determined that the highest number of plants per square metre 27.33 was achieved from sowings carried out on 18 May with an in-row spacing of 8 cm. In addition to the higher seed rate used for sowing at 8 cm between rows, the favourable conditions for seed germination and growth prevailing on 18 May proved conducive to the development of the beans, and it was found that the number of plants per square metre was higher than in the other sowing treatments. It has been noted that low soil temperatures in spring cause a delay in emergence in beans, a cold-sensitive species; consequently, the germinating seeds are exposed to pathogens and pests in the soil for a longer period, leading to seed rot and a reduction in the emergence rate in other words, a decrease in the number of plants per square metre. (Dickson and Boetger 1984; Mohammed et al. 1988).

Plant Height: In this study, when examining the effect of different sowing densities and sowing times on plant height in the Alberta bean variety, it was found that the effect of sowing time was very significant. In this study, it was determined that the interaction between sowing time and sowing density had a negligible effect on plant height. Whilst the effect of sowing time on plant height was found to be negligible in the same study, the effect of sowing density on plant height was found to be statistically highly significant ($p \leq 0.01$).

Table 5. Results of the analysis of variance for plant height (cm) in the Alberta bean variety under different sowing densities and sowing times

	S D	Sum of ~	Mean of ~	F value	Significance
Repetition	2	24.04	12.02	0.67	
Sowing Time (ST)	4	8993.87	2248.46	125.84	0.00**
Error 1	8	142.93	17.86		
Sowing Density (SD)	4	1818.84	454.71	12.74	0.00**
ST × SD Int.	16	254.84	15.92	0.44	
Error 2	40	1426.83	35.67		
General	74	12661.38			

CV (%): 12,37; * Significant at the $P \leq 0.05$ level, ** Significant at the $P \leq 0.01$ level,

In the study, the beans reached their maximum plant height in the crops sown on 18 May. The average plant height was recorded as 58.44 cm. It was determined that the beans were significantly affected by both high and low temperatures during their growth period. The beans sown on 18 May emerged uniformly within 10–12 days. The emergence period coincided with a time when ideal air temperatures for bean growth were present, allowing the plants to develop under the healthiest conditions and reach their maximum plant height. Studies on beans indicate that plant height varies within a range of 17.67–310 cm. The fact that the figures fall within such a wide range is significantly influenced by whether the beans are dwarf or climbing varieties (Bozoğlu and Sözen, 2007; Ülker ve Ceyhan, 2008; Pekşen, 2005; Anlarsal et al., 2000; Kahraman et al., 2013). Pekşen (2005) reported that plant height is influenced not only by genetic structure but also by environmental conditions.

Depending on the sowing date, the lowest plant height was observed in beans sown on 15 June. It was found that plant height measured 32.97 cm in plots sown on these dates. In the beans sown on 15 June, exposure to high air temperatures during the emergence period and the subsequent vegetative growth period caused the plants to experience heat stress, resulting in stunted plant height.

Table 6. Means for plant height (cm) of the Alberta bean variety under different sowing densities and sowing times, and results of the Duncan test for comparisons between means

Sowing Times	Planting Density					
	8 cm	10 cm	12 cm	14 cm	16cm	Avg.
20 April	66.70	58.26	54.46	56.26	54.93	58.12 ab
4 May	61.46	62.13	51.66	52.53	45.80	54.72 b
18 May	64.66	60.13	60.63	56.50	50.30	58.44 a
1 June	46.46	39.63	37.20	33.26	29.26	37.16 c
15 June	39.90	35.93	33.06	29.26	26.70	32.97 d
Avg.	55.84 a	51.22 b	47.40bc	45.56 cd	41.40 d	

*The difference between means denoted by the same letter is not significant (Duncan's test, $p \leq 0.05$)

The study found that sowing density has a significant effect on plant height. Measurements revealed that the highest plant height, 55.84 cm, was recorded in plots sown at a density of 8 cm. It is thought that this is due to the

intense competition between the beans as they vie for sunlight. In plots sown at a row spacing of 10 cm—which is close to the 8 cm row spacing—a plant height of 51.22 cm was recorded. It was observed that plant height decreased as the in-row spacing increased; in beans sown at 12 cm and 14 cm in-row spacing, plant heights of 47.40 cm and 45.56 cm, respectively, were recorded. In the plots where sowing was carried out at a row spacing of 16 cm—and where the shortest plant height recorded was 41.40 cm—it was determined that the reason for the shorter plant height was that the distance between plants was greater than that at a sowing density of 8 cm; consequently, the plants developed with less competition for sunlight.

Height of the First Pod: The effect of five different sowing times and five different sowing densities on the height of the first pod in beans was investigated. In this study, the effect of sowing time on the height of the first pod was found to be highly significant at the $p \leq 0.01$ level. It was determined that the interaction between sowing time and sowing density had a highly significant effect.

In this study, which found that five different sowing dates had a significant effect on the height of the first pod, it was determined that the greatest height of the first pod was obtained from the plots sown on 18 May. It was found that the results from the plots sown on 20 April and 1 June were similar. In the plots sown on 1 June, the initial pod height was measured at 7.21 cm, whilst in the plots sown on 20 April, it was 7.13 cm. The first pod height obtained from beans sown on 4 May was determined to be 6.69 cm. The sowing date yielding the shortest first pod height was identified as 15 June. The first pod height of beans from plots sown on this date was determined to be 6.17 cm. It was found that the first pod height in beans is directly proportional to plant height. The reason why the sowing time had a significant effect on the height of the first pod was determined to be the different climatic conditions experienced at each sowing time. It has been established that the reason the height of the first pod was greater in the plots sown on 20 April, 4 May and 18 May compared to the other plots was that the temperatures during this period were more favourable for the development of the beans. It was found that rising temperatures adversely affected vegetative growth in the early stages of the beans, causing the plants to become stunted. (Bozoğlu, 1995) determined the average height of the first pod in dwarf dry bean genotypes grown at seven

different locations to be 13.6 cm; he reported that, as the heritability of this trait was as low as 57 per cent, the influence of environmental factors was high.

Table 7: Results of the analysis of variance for the height of the first pod (cm) under different sowing densities and sowing times

	S D	Sum of	Mean of	F value	Significance
Repetition	2	3.75	1.88	2.51	
Sowing Time (ST)	4	47.60	11.90	15.93	0.0007**
Error 1	8	5.97	0.74		
Sowing Density (SD)	4	30.77	7.69	17.43	0.0000**
ST × SD Int.	16	18.75	1.17	2.65	0.0063**
Error 2	40	17.65	0.44		
General	74	124.52			

CV (%): 9,28;
* Significant at the P ≤ 0.05 level, ** Significant at the P ≤ 0.01 level

Table 8: Means for the height of the first pod (cm) under different sowing densities and sowing times, and results of the Duncan test for comparisons between means

Sowing Times	Planting Density					
	8 cm	10 cm	12 cm	14 cm	16cm	Avg.
20 April	7.56cdef	7.03efgh	6.93efgh	7.26defg	6.86fgh	7.13
4 May	7.33cdefg	7.60cdef	7.20efg	5.46ijk	5.86hij	6.69b
18 May	9.56 a	8.96 ab	8.56abc	8.16bcde	7.56cdef	8.56
1 June	8.50abcd	6.63fghı	7.46cdef	8.20bcde	5.26jk	7.21
15 June	7.13efgh	6.43fghıj	6.50fghıj	6.13ghıj	4.66 k	6.17
Avg.	8.02 a	7.33 b	7.33 b	7.04 b	6.04 c	

*The difference between means denoted by the same letter is not significant (Duncan's test, p ≤ 0.05)

The study found that the average height of the first pod ranged from 4.66 cm to 9.56 cm. Gülümser and Zeytun (1988) determined that in the beans they cultivated in the Çarşamba Plain, the number of seeds per pod ranged from 3.0 to 5.6, the height of the first pod ranged from 6.0 to 13.1, and the protein content was 25.59 per cent. When the effect of sowing density on the height of the first pod in beans was examined in the study, it was found that the height of the first pod varied between different sowing densities. It was determined that the lowest height of the first pod, at 6.04 cm, was obtained from plots sown at a row spacing of 16 cm. Plots sown at 14 cm between-row spacing yielded an initial pod height of 7.04 cm. Plots sown at 10 cm and 12 cm between-row

spacing yielded the same initial pod height, which was determined to be 7.33 cm for these beans. The greatest effect of sowing density on the first pod height in beans was observed in plots sown at 8 cm between-row spacing. In a study conducted by Gomaa et al. (1981) using the William and Clark varieties between 1978 and 1979, with plant densities of 57,000, 71,000, 101,000 and 141,000 plants/ha; they found that whilst increasing plant density increased seed yield, plant height and the height of the first pod set, it reduced the number of pods per plant, the number of seeds per pod, the weight of 100 seeds and the number of side shoots per plant. The lowest first pod height in beans, at 8.02 cm, was recorded in the plots where a planting density of 8 cm was applied. In this study, which found that different sowing densities and sowing dates had a significant effect on the first pod height of the 'Alberta' bean variety, the highest first pod height was recorded in plots sown on 18 May at a row spacing of 8 cm. In these plots, the first pod height of the beans was determined to be 9.56 cm. It was found that the shortest initial pod height was obtained from beans sown on 15 June at a row spacing of 16 cm. In these plots, the average initial pod height of the beans was determined to be 4.66 cm. According to the results of the trial, the height of the first pod in beans was found to be directly proportional to the sowing density. It was observed that the height of the first pod decreased with both dense and sparse sowing, as well as with late and early sowing.

Number of Main Stems: When the effect of different sowing densities and sowing times on the number of main stems in beans was examined, it was found that sowing time had a significant effect. The same table also showed that sowing density had a significant effect on the number of main stems. In this study, it was found that the interaction between sowing density and sowing time had a statistically significant effect ($p \leq 0.05$) on the number of main stems in beans.

Table 9: Results of the analysis of variance for the number of main branches in the Alberta bean variety under different planting densities and planting times

	S D	Sum of Squares	Mean of Squares	F value	Significance
Repetition	2	0.99	0.49	13.16	0.0129
Sowing Time (ST)	4	70.87	17.71	467.89	0.0000**
Error 1	8	0.30	0.03		
Sowing Density (SD)	4	2.82	0.70	3.69	0.0119*
ST × SD Int.	16	6,73	0,42	2,19	0,0220*
Error 2	40	7,65	0,19		
General	74	89,38			

CV (%): 11,75; * Significant at the P ≤ 0.05 level, ** Significant at the P ≤ 0.01 level

Tablo 10: Means and Duncan test results regarding the number of primary branches in the Alberta bean variety under different sowing densities and sowing times.

Sowing Times	Planting Density					
	8 cm	10 cm	12 cm	14 cm	16cm	Avg.
20 April	4.43 cdef	4.23 def	4.13 def	5.20 abc	5.53 a	4.70 a
4 May	3.93 ef	4.73 abcde	4.73 bcde	4.86 abcd	5.33 ab	4.72 a
18 May	3.73 f	4.23 def	4.13 def	4.36 def	3.73 f	4.04 b
1 June	2.46 g	2.86 g	2.70 g	2.33 g	2.70 g	2.61 c
15 June	2.50 g	2.70 g	2.43 g	2.33 g	2.66 g	2.52 c
Avg.	3.41 c	3.75 ab	3.62 bc	3.82 ab	3.99 a	

*The difference between means denoted by the same letter is not significant (Duncan's test, p ≤ 0.05)

The number of main stems varied according to the planting date, and it was found that the average number of main stems decreased as the planting date was delayed. The highest number of main stems was recorded in the plots planted on 4 May and 20 April, at 4.72 and 4.70 respectively. It was found that there were 4.04 main stems in the beans sown on 18 May. The number of main stems in the plots sown on 1 June was found to be 2.61. The lowest number of main stems, at 2.52, was recorded in the plots where the beans were sown on 15 June, the final sowing date of the trial. The study found that the number of main branches was higher in plants sown at 16 cm row spacing compared to other sowing densities. The highest number of main branches was observed in plants sown at 16 cm row spacing, with 3.99 main branches per bean plant. It was found that a lower number of beans per unit area enabled the plant to utilise water and nutrients more effectively. Consequently, it was observed that the

plant was able to branch out without competition or stress, thereby achieving the maximum number of main branches. In the plots sown at the lowest spacing of 8 cm between rows, the number of main branches was found to be 3.41. In these plots, where the lowest number of main stems was observed, the higher number of plants per unit area compared to plots with other planting densities indicated that the beans were unable to utilise plant nutrients, fertiliser and water sufficiently. It was found that the number of main stems obtained from planting at 10 cm row spacing was 3.75. The number of main stems obtained from plots where a 12 cm inter-row spacing was used was lower than that from plots with a 10 cm inter-row spacing; it was found to be 3.62. After the number of main stems obtained from the 16 cm spacing, the highest number of main stems was recorded in the plots where a 14 cm inter-row spacing was used. It was found that the number of main branches was higher in sparse sowings carried out at early dates compared to dense sowings carried out at later dates. The highest number of main branches was recorded in plots sown on 20 April at row spacings of 16 cm. An average of 5.53 main branches were obtained from the beans in these plots.

Grain Yield: The study found that sowing time had a significant effect on grain yield (Table 11). It was determined that sowing density had a statistically significant effect ($p \leq 0.01$) on grain yield. Furthermore, the study found that the interaction between sowing time and sowing density had a statistically significant effect ($p \leq 0.05$) on grain yield.

Table 11: Results of the analysis of variance for grain yield in relation to different sowing densities and sowing times

	S D	Sum of S	Mean of S	F value	Significance
Repetition	2	932.58	466.29	1.05	0.3917Ns
Sowing Time (ST)	4	397161.92	99290.48	224.86	0.0000**
Error 1	8	3532.48	441.56		
Sowing Density (SD)	4	93443.62	23360.90	31.45	0.0000**
ST × SD Int.	16	22984.27	1436.51	1.93	0.0460*
Error 2	40	29709.60	742.74		
General	74	547764.50			

CV (%): 15.62; * Significant at the $P \leq 0.05$ level, ** Significant at the $P \leq 0.01$ level, Ns Not significant

In the study, when the effect of sowing time on grain yield was examined, it was found that different sowing times resulted in significant differences in grain yield in beans. The sowing time yielding the highest grain yield was identified in the plots sown on 4 May. The grain yield from these plots was found to be 253.7 kg. It was found that a grain yield of 242.2 kg was obtained from the beans in the plots sown on 20 April. It was noted that the tall plant height of the beans sown on 20 April had a positive effect on grain yield. It was found that problems with emergence in the late sowings, as well as adverse developments during the flowering and pod-setting stages caused by high-temperature stress, had a negative impact on grain yield. It was found that the grain yield from sowings carried out on 18 May was higher than that from sowings carried out in June. It was found that a grain yield of 199.9 kg was obtained from the plots sown on 18 May. It was found that the lowest grain yield was obtained from sowings carried out on 15 June. It was noted that plant desiccation due to high temperatures occurred during this sowing period, when the lowest grain yield of 81.93 kg/da was recorded.

According to Akçin (1974), research conducted over a two-year period in 1969 and 1970 on a total of 16 dry bean genotypes in the Erzurum ecosystem revealed that the most suitable sowing date for maximising variety yields was 15 May, and the most suitable row spacing was 20 cm. In the variety yield trials, the varieties that produced the highest seed yields had earlier germination, flowering and vegetative growth periods; these varieties completed their vegetative growth in 99–106 days, whilst those with lower yields took 113–122 days to do so. The protein content of high-yielding varieties was lower than that of other varieties. In a study conducted by Pekşen (2005) in Samsun in 2002 and 2003, aimed at determining the relationships between grain yield and yield-related traits in certain bean (Phaseolus vulgaris L.) genotypes, as well as the direct and indirect effects of these traits on grain yield, it was reported that there were positive and significant correlations between grain yield and plant height, and positive and highly significant correlations between grain yield and the number of pods, the number of seeds per plant, pod length, stem yield and the height of the first pod. It was found that as the sowing date advanced, there was a proportional decrease in grain yield.

Akçin (1988) stated that the time taken for seeds to germinate and emerge from the soil surface is an important factor affecting yield and quality in field

crop production. In his study aimed at determining the effects of temperature on the germination of dry beans, the researcher reported that optimum germination of dry bean seeds was achieved when the soil temperature was 18–20 °C; that high and low humidity levels led to reduced yield, flower drop and the spread of diseases; and that relative humidity should be around 50 per cent for a good seed yield. When examining the effect of sowing density on grain yield in the study, it was found that grain yield decreased as the in-row spacing between beans increased; the highest grain yield, at 224.8 kg/da, was recorded in plots sown at 10 cm in-row spacing. The lowest grain yield was recorded at 127 kg/da in plots where a 16 cm in-row spacing was applied. Yields of 144.6 kg/da were recorded in plots with a 14 cm in-row spacing, and 178.4 kg/da in plots with a 12 cm in-row spacing. Chatterjee and Som (1991) reported that in their study conducted in West Bengal between 1984 and 1987, using three different planting densities (40 × 10, 40 × 15, and 40 × 20 cm), the highest grain yield (2.88 t/ha) was obtained from plots where a 40 × 10 cm planting density was applied.

Tablo 12: Means for grain yield at different sowing densities and sowing times, and Duncan test results comparing the means.

Sowing Times	Planting Density					
	8 cm	10 cm	12 cm	14 cm	16cm	Avg.
20 April	276.7	304.8 b	248.7 cde	197.7 fghı	183.0 ghı	242.2 a
4 May	259.8	358.3 a	249.3 cde	208.3 efgh	192.9 fghı	253.7 a
18 May	227.2	237.5 cdef	220.8 defg	160.8 hıj	153.3 ıj	199.9 b
1 June	111.0	120.7 jk	98.33 klm	85.33 klm	57.0 lm	94.47 c
15 June	112.5	102.8 kl	75.0 klm	70.67 klm	48.67 m	81.93 c
Avg.	197.4 b	224.8 a	178.4 b	144.6 c	127.0 c	

*The difference between means denoted by the same letter is not significant (Duncan's test, $p \leq 0.05$)

Deshpande et al. (1995), in their study on beans in India using three different row spacings (10, 15 and 20 cm), reported that the highest grain yield per unit area (272 kg/da) was obtained with a row spacing of 10 cm. It was found that a grain yield of 197.4 kg/da was obtained from plots where an 8 cm in-row spacing was applied. It was observed that the higher number of plants per unit area resulted in a higher grain yield in dense plantings compared to sparse plantings. In another study, Singh et al. (1996) conducted a sowing

density trial on beans using row spacings of 25 cm and 30 cm, combined with in-row spacings of 10 cm and 15 cm; they found that the highest grain yield was obtained from the 30 cm × 10 cm sowing density. The study also found that the interaction between sowing time and sowing density had a significant ($p \leq 0.05$) effect on grain yield. The highest grain yield, at 358.3 kg/da, was recorded in plots sown on 4 May at a row spacing of 10 cm. The study found that the lowest grain yield was obtained from plots sown on 15 June at a row spacing of 16 cm. The grain yield from these plots was found to be 48.67 kg/da. In their study conducted under Van–Gevaş conditions to determine the optimal sowing time for a sugar bean variety, Çiftçi and Allahverdi (2001) found that sowing times had a significant effect on grain yield per unit area and certain yield components, and concluded that the best sowing time was in early May. In the present study, it was found that grain yield decreased in cases of late and sparse sowing. In the present study, it was found that grain yield decreased in cases of late and sparse sowing.

Hundred-Seed Weight: In the study, when the effects of different sowing densities and sowing times on hundred-seed weight were examined, it was found that both sowing time and sowing density had a significant effect. It was also found that the interaction between sowing time and sowing density had a significant effect.

In this study, it was found that the highest hundred-seed weight was obtained from the plots sown on 18 May. The hundred-seed weight of the beans from these plots was determined to be 33.17 grams. The hundred-seed weight was found to be 31.81 grams for the plots sown on 4 May and 29.81 grams for those sown on 20 April. It was found that the lowest 100-seed weight in the study was obtained from the plots sown on 1 June and 15 June. The 100-seed weight was determined to be 25.43 grams for the plots sown on 15 June and 24.69 grams for those sown on 1 June.

Table 13: Results of the analysis of variance for the weight of 100 seeds under different sowing densities and sowing times

	S D	Sum of	Mean of	F value	Significance
Repetition	2	50.15	25.07	1.81	0.2238 Ns
Sowing Time (ST)	4	859.05	214.76	15.54	0.0008**
Error 1	8	110.51	13.81		
Sowing Density	4	71.45	17.86	3.34	0.0187*
ST × SD Int.	16	284.39	17.77	3.33	0.0010**
Error 2	40	213.43	5.33		
General	74	1589.00			

CV (%): 7.97; * Significant at the P ≤ 0.05 level, ** Significant at the P ≤ 0.01 level, Not significant

Table 14: Means for hundred-seed weight of the Alberta bean variety under different sowing densities and sowing times, and results of the Duncan test for comparisons between means

Sowing Times	Planting Density					
	8 cm	10 cm	12 cm	14 cm	16cm	Avg.
20 April	29.37 cde	30.73 bcd	30.40bcd	29.43 cde	29.13 cde	29.81 b
4 May	30.10 bcd	39.03 a	28.87 cde	30.83 bcd	30.20 bcd	31.81 ab
18 May	32.20 bcd	34.00 b	33.17 bc	34.07 b	32.43 bcd	33.17 a
1 June	25.10 efg	25.43 efg	25.10 efg	24.23 fg	23.60 g	24.69 c
15 June	21.57 g	23.60 g	24.23 fg	28.27 def	29.46 cde	25.43 c
Avg.	27.67 b	30.56 a	28.35 b	29.37 ab	28.97 ab	

*The difference between means denoted by the same letter is not significant (Duncan's test, p ≤ 0.05)

In this study, which highlighted the significant effect of sowing density on beans, the highest 100-seed weight of 30.56 grams was recorded in plots where a row-to-row spacing of 10 cm was used. Among the other inter-row spacing treatments, the lowest 100-seed weight of 27.67 grams was recorded in the plots where the densest inter-row spacing of 8 cm was applied. A 100-seed weight of 28.35 grams was recorded in the plots where an inter-row spacing of 12 cm was used. In the study, the hundred-seed weight of beans sown at the widest inter-row spacing of 16 cm was observed to be 28.97 grams, whilst that of beans sown at 14 cm inter-row spacing was determined to be 29.37 grams.

CONCLUSIONS AND RECOMMENDATIONS:

Although the results of this study are based on a single year, we believe that 1 May is the most suitable sowing date for the region; furthermore, we believe that sowing beans at this time, with a row spacing of 40 cm and a spacing of 10 cm within the row, will result in high grain yields in the region. We also recommend that the study be repeated over two or more years in order to obtain more meaningful results.

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CHAPTER 6

INTEGRATING ONE HEALTH INTO PLANT HEALTH BSc EDUCATION

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INTRODUCTION: Plant Health in the One Health Era

Global demographic trends and the consequent intensification of resource demands pose significant challenges, calling for a comprehensive paradigm that links crop production ecosystems to public health. Projected population growth and its domino effects on food security underpin long-term strategic planning. Under median demographic scenarios, the global population, which has grown linearly since 1950, is expected to maintain this trajectory until around 2080, achieving roughly 10.3 billion - an increase of about 2 billion from current levels. High-variant fertility projections also suggest that the global population could reach 14 billion by the end of the century (UN DESA, 2024).

This population concentration accelerates the spread, mutation, and emergence of more virulent infectious agents in a globalized context, resulting in major threats to ecological resilience (Erdoğan et al., 2024). Addressing these complex ecological and social risks necessitates the effective preservation of plants, as primary ecosystem producers, and their associated microbiomes. The inseparable connections among plant health, food safety, and human well-being are central to the "One Health" framework (Lorenzini & Nali, 2025).

Data from the Food and Agriculture Organization indicate that plant diseases cause devastating annual crop production losses of 20%–40% globally (FAO, 2022). Consequently, sustainable crop production, the mitigation of pollution risks, the provisioning of ecosystem services, and the curtailment of resistance risks associated with agricultural inputs is directly determined by plant health parameters (Lorenzini & Nali, 2025). Guaranteeing long-term food security and nutritional sustainability fundamentally relies on the safeguarding of plant resources. Because plants form the foundation of food security and human nutrition, they play a vital role in One Health as an integrated, unifying approach that aims to sustainably balance and optimize the health of people, plants, animals, and ecosystems (FAO, 2026).

These multidimensional dependencies mandate a transdisciplinary, integrated action plan designed to sustainably optimize human, animal, plant, and environmental health (FAO, UNEP, WHO, & WOAH, 2022; OHHLEP, 2022). By presenting a holistic paradigm that acknowledges the intrinsic interdependence of these components, the One Health approach mobilizes

multisectoral coordination to address joint challenges ranging from food security to the climate crisis (FAO, 2026; OHHLEP, 2022).

The implementation of sustainable protection strategies in crop production ecosystems, aligned with the principles of Integrated Pest Management (IPM), plays a decisive role in safeguarding environmental and human health. Establishing food security and ecological robustness depends on conducting multidimensional cost-benefit analyses in plant health practices. The rational management of chemical inputs and the successful execution of resistance-alleviation strategies in crop production areas are of paramount importance for preserving holistic ecosystem health (Hoffmann et al., 2022).

Translating ecological priorities at the field level into sustained crop production practices depends heavily on the quality and conceptual training of the professionals overseeing these agroecosystems. Achieving these objectives entails comprehensive pedagogical reform to equip future plant health specialists with advanced ecological and ethical competencies (Akan & Şahin, 2024).

Achieving these educational and ecological objectives requires a structural paradigm shift in higher education through the establishment of a dedicated, standalone 14-week undergraduate course titled “Plant Health in One Health”. Dispersing core One Health concepts across multiple disjointed existing modules carries the severe pedagogical risk of fragmenting student learning, preventing undergraduates from internalizing the unified, systemic nature of agroecological health. Institutionalizing a comprehensive, independent course provides the necessary structural platform with explicit learning outcomes, enabling students to systematically synthesize phytopathology, toxicological risk assessment, food safety governance, and public health policies into an integrated analytical framework (Akan & Şahin, 2024).

1. GLOBAL MODELS OF ONE HEALTH EDUCATION

Proactively managing global plant health and biosecurity risks demands integrated educational models that systematically rise above traditional disciplinary silos in higher education. Pioneering global institutions-specifically The Ohio State University, the University of Tennessee, and North Carolina State University-established invaluable early frameworks by

designing standalone minor or elective programs that unify plant health under the One Health umbrella (The Ohio State University, 2026). Enabled by such foundational curricula, undergraduate students comprehend clinical-level interactions among human, animal, plant, and environmental systems, creating a highly valuable reference point for institutionalizing cross-disciplinary collaboration. Despite these pedagogical milestones, the direct and immediate integration of standalone programs into existing academic structures remains heavily constrained by institutional bureaucracy and a persistent shortage of specialized faculty.

Establishing a dedicated, standalone integrated curriculum titled “Plant Health in One Health” represents the only pedagogically sound framework capable of fostering comprehensive systems thinking. Fragmenting these interdisciplinary topics into minor secondary units within existing courses undermines learning cohesion, reducing holistic health principles to isolated technical examples. Resolving the environmental, economic, and community dimensions of sustainability within crop production ecosystems requires centering these complex, interdependent dynamics directly within a single, unified curriculum. Through constructivist pedagogy embedded in a dedicated course, crop protection undergraduates transform from technical operators focused strictly on chemical-based interventions into strategic decision-makers capable of managing the tripartite pillars of ecological sustainability (Akan et al., 2024a). Consequently, higher education institutions must prioritize the immediate creation of fully independent, standalone courses rather than relying on piecemeal curricular overlays. Modern international health emergencies cannot be resolved through adjacent, loosely coordinated multidisciplinary cooperation; they require the deliberate creation of unified knowledge and management systems. Under this vision, One Health is quickly evolving into a transdisciplinary framework that unites information professionals and strategic decision-making mechanisms (Göncüoğlu, 2026). This educational transformation establishes a shared scientific lexicon across human, veterinary, environmental, and plant health disciplines, thereby constructing a resilient, integrated shield to safeguard environmental balance against devastating and destructive systemic pressures (Özkul, 2026).

2. INTEGRATED CURRICULUM DESIGN AND STANDALONE COURSE STRUCTURE

Addressing emerging biosecurity threats in undergraduate crop production curricula requires abandoning fragmented instructional approaches in favor of a dedicated, standalone holistic pedagogical framework titled “Plant Health in One Health”. While administrative inertia has historically led institutions to consider temporary overlay designs, relying on dispersed modules weakens the pedagogical integrity of transdisciplinary training. Institutionalizing a unified, standalone course ensures that core biosecurity threats, ecotoxicological risks, and interdisciplinary case studies are systematically connected within a robust analytical framework (Akan et al., 2024a; Akan et al., 2024b). Fragmenting One Health principles across disconnected, existing courses prevents undergraduates from internalizing the unified, systemic nature of agroecological health. Therefore, higher education curricula must be structured around a dedicated 14-week syllabus centered on holistic risk assessments, vector dynamics, mycotoxin governance, and biosecurity policies (FAO, UNEP, WHO, & WOAH, 2022). This evolutionary transition avoids ad hoc implementation and guarantees that students acquire a robust, non-fragmented capability to analyze multi-tiered biological threats, proceeding strictly within an integrated framework aligned with institutionalization standards established by international governing bodies and environmental policymakers (FAO, UNEP, WHO, & WOAH, 2022).

3. MYCOTOXINS AND FOOD SAFETY REGULATIONS

Within crop production ecosystems, the pre- and post-harvest management of phytopathogens serves as a strategic biosecurity barrier. Beyond merely mitigating yield and quality losses, this intervention directly prevents the accumulation of carcinogenic mycotoxins that threaten human and animal health. Secondary metabolites synthesized by ubiquitous fungal genera-specifically *Aspergillus*, *Penicillium*, and *Fusarium*-can readily infiltrate food and feed supply chains, posing widespread ecotoxicological and clinical risks to consumers (Papri et al., 2022).

Maintaining robust plant health across open-field and protected crop production systems through target-oriented stress management represents a primary, cost-effective strategy to suppress post-harvest fungal proliferation

and the subsequent accumulation of devastating toxins. Within undergraduate crop protection curricula, the proper adoption of preventive plant health protocols and pre-harvest host resistance mechanisms must be systematically integrated with a dedicated focus on these clinical and toxicological consequences (Strosnider et al., 2006).

Contamination of botanical raw materials destined for animal feed by toxicogenic fungal species drives substantial economic losses in the livestock sector while indirectly facilitating zoonotic disease transmission by inducing host immunosuppression. Developing crop protection professionals must comprehend these interrelated economic and physiological damage dynamics operating at the plant–animal health interface to design targeted mitigation strategies (Bezerra da Rocha et al., 2014; Zaki et al., 2012).

From a global food security and biosecurity perspective, managing sclerotia-forming fungal pathogens, such as *Claviceps purpurea* in pre-harvest cereals, represents a persistent historical and contemporary toxicological challenge spanning human and veterinary medicine (Klotz, 2015; Schardl et al., 2006). Suppressing this pathogen must transcend isolated plant pathology modules, finding integration within food safety legislative frameworks. Although strict regulatory thresholds for processed cereal products have successfully minimized human dietary exposure, recurring clinical issues in livestock from contaminated feed highlight the necessity of continuous crop protection interventions (European Union, 2021, 2023).

Mitigating mycotoxin contamination requires a comprehensive surveillance network that spans from Good Agricultural Practices (GAP) in crop production fields to precise temperature and humidity controls during storage, coupled with strict compliance with internationally accepted regulatory limits. Integrating food safety modules into the undergraduate crop protection curriculum is of paramount importance for equipping future professionals with these essential regulatory and administrative competencies (EFSA, 2026).

4. ENVIRONMENTAL RISKS OF SYNTHETIC FUNGICIDES

The environmental fate and dynamic cycling of chemical pesticides applied to suppress insect pests, pathogens, and weeds in crop production landscapes carry profound ecotoxicological risks, driving non-target exposure

and biodiversity loss. Plant protection professionals face the major challenge of managing the environmental dissemination pathways and residual lifetimes of these inputs across soil, aquatic, and atmospheric compartments. Indiscriminate chemical applications create persistent residues that directly threaten the health of human populations, wildlife cohorts, and broader ecosystem health (Erdoğan et al., 2024).

Systemic triazole fungicides, widely deployed to suppress fungal pathogens in crop production systems, trigger hazardous cross-resistance mechanisms in species of vital significance to human clinical medicine. Uncontrolled azole use in agricultural, veterinary, and clinical settings has driven the development of resistance, leading to a subsequent loss of efficacy against over 30 plant pathogens in more than 60 countries (FRAC, 2025). This environmental selection pressure signifies a critical global crisis, requiring the successful execution of coordinated surveillance protocols bridging medical and phytoprotection specialists.

Intense triazole exposure within crop production landscapes directly mediates the evolutionary selection whereby opportunistic environmental fungi acquire resistance to clinical azoles before encountering human patients. This pathway illustrates the indirect, highly concerning impact of agricultural interventions on public health: resistance patterns selected by crop protection azoles cross over to compromise medical triazoles, elevating clinical anxieties to a critical level (Snelders et al., 2012). Widespread fungicide applications in crop production environments directly or indirectly select for azole-resistant phenotypes in clinically significant genera, including *Aspergillus*, *Fusarium*, and *Candida* (Ribas et al., 2016). Sharing identical environmental niches with plant-associated fungi, *Aspergillus fumigatus* is directly exposed to the selective pressure of agricultural azoles, with contemporary studies establishing a correlation between localized resistance frequencies and residual fungicide concentrations in soil (Cao et al., 2021).

Unregulated use of clinically significant antibiotics to suppress bacterial diseases in crop production environments, particularly pome fruit orchards, accelerates the accumulation of environmental reservoirs of resistance and drives the selection of resistant variants in both human and plant pathogens. Streptomycin, a mainstay of human tuberculosis therapy, continues to be deployed in horticulture to manage fire blight, caused by the bacterial pathogen

Erwinia amylovora, on host fruit trees. Due to the emergence of extensive resistance in clinical and farming settings, the use of streptomycin has been heavily restricted or entirely banned in European countries (Davies & Wright, 1997; McManus & Jones, 1994). Beyond temperate orchards, paddy rice systems represent another strategic crop production sector in which agricultural antibiotics are routinely applied, expanding the environmental resistome (Taylor & Reeder, 2020).

Ecological degradation associated with phytoprotection is not restricted to active chemical residues. The post-application management of pesticide container packaging, classified as hazardous waste, requires a holistic stewardship model that connects environmental protection and circular economy principles. Within the frameworks of "zero-waste" initiatives and sustainable recycling paradigms, establishing systematic collection and disposal programs for empty crop protection packaging is of paramount importance to secure global crop production while protecting ecological health, in accordance with overarching One Health objectives (Erdoğan et al., 2024).

5. GOOD AGRICULTURAL PRACTICES (GAP) IN CROP PRODUCTION

Reducing biological and chemical contaminants throughout pre- and post-harvest stages relies heavily on the strict application of Good Agricultural Practices (GAP). Within higher education, integrating these operational standards into hands-on practical components within the dedicated One Health course enables plant protection undergraduates to project theoretical principles directly onto active crop production fields.

Simultaneously, implementing GAP alongside optimized post-harvest storage technologies across open-field and protected crop production systems represents a critical intervention. This integrated strategy secures food safety by systematically arresting devastating fungal proliferation and subsequent mycotoxin accumulation, thereby establishing a robust baseline for compliance with stringent global regulatory standards.

Rather than reducing GAP guidelines to rote memorization through static compliance checklists, constructivist pedagogy requires students to experience these protocols first-hand at model farming enterprises. Engaging directly with operational farms exposes future phytoprotection professionals to the nuances

of real-world auditing, precise record-keeping, and the administrative governance of certification pathways.

Endowing graduates with a systemic understanding of ecological sustainability, pesticide residue dynamics, and global certification frameworks is of paramount importance for their effective implementation within the contemporary crop production sector. Aligning this training with modern legislative instruments, such as the European Union Directives that prioritize Integrated Pest Management (IPM) and biological alternatives, represents a highly dynamic protective mechanism for safeguarding both human communities and environmental health (European Union, 2009a, 2009b).

6. INSECT VECTORS AND HUMAN PATHOGENS

The infiltration of human pathogens into crop production environments through irrigation water or organic soil amendments represents far more than a passive environmental contamination event. The active dissemination of these enteric microorganisms onto foliar surfaces-mediated by the feeding, grooming, and movement of insect vectors within crop production agroecosystems-directly compromises food safety. The structural hygiene of the crop production environment and the targeted mitigation of botanical contamination thus emerge as critical nodes governing produce safety across the entire "farm-to-fork" continuum.

Vector insects mechanically or biologically harbor and transfer human pathogens from contaminated agricultural water to plant tissues. This capacity, coupled with the "dual-life" adaptive nature of enteric pathogens that allows survival within both vegetative hosts and animal systems, creates a complex, multidimensional biosecurity threat that transcends the traditional boundaries of phytoprotection and basic entomology (Teplitski & de Moraes, 2018).

Within undergraduate entomology and plant pathology modules, tracing the transmission pathways of lethal strains, such as Shiga toxin-producing *Escherichia coli* (STEC), through insect vectors and the agricultural environment is of paramount importance to prevent widespread foodborne outbreaks. Systemic tracking and risk profiling of these high-risk contamination events in fresh horticultural produce are heavily documented by international regulatory authorities (EFSA, 2011).

Entomological curricula must extend beyond classical vector-virus relationships to address with scientific clarity whether plant-associated viruses can cross kingdom boundaries to pose direct pathogenic risks to human and veterinary health. Incorporating these transdisciplinary concepts is vital for establishing a unified biosecurity lexicon across medical, veterinary, and crop protection disciplines (Balique et al., 2015).

Safeguarding plant health and managing arthropod vectors in crop production agroecosystems provides a primary defense mechanism. Beyond securing yields, the effective implementation of entomological vector controls directly shields public health by suppressing pathogen reservoirs in the food chain, reducing non-zoonotic vector loads, and minimizing the selection pressures that accelerate antimicrobial resistance.

7. PHYTOPATHOGENS AS OPPORTUNISTIC HUMAN THREATS

Although entomopathogenic agents used in biological pest control within sustainable crop production ecosystems offer environmentally friendly solutions, they can directly infect humans, triggering clinical infection when biosecurity thresholds are breached. Fungal taxa widely deployed as entomopathogenic agents-including *Beauveria bassiana* and *Metarhizium anisopliae*-can manifest pathogenicity in clinical settings, thereby inducing opportunistic diseases (Tucker et al., 2004). This capacity of agricultural agents within crop production environments to overcome physiological barriers in mammalian hosts represents a transdisciplinary evolutionary phenomenon, indicating that host ranges are not static and necessitating vigilant surveillance across the medical–agricultural interface. While plant and animal pathogens typically colonize divergent taxonomic lineages, certain phytopathogens cross these evolutionary boundaries to infect humans and animals in rare, highly documented instances (Kim et al., 2020).

Saprophytic or weakly parasitic fungal agents colonizing horticultural and industrial crop production fields can trigger severe clinical complications, presenting as deep-tissue mycoses in immunocompromised transplant recipients. The phytopathogen *Alternaria infectoria*, the causative agent of blossom blight in guayule (*Parthenium argentatum*), has been directly linked to the development of phaeohyphomycosis in a renal transplant patient (Halaby

et al., 2001). Common soilborne pathogens with broad botanical host ranges that cause wilt diseases in crop production systems emerge as opportunistic agents in clinical medicine, targeting nail tissues and driving systemic invasive circulatory infections in immunocompromised patients. Beyond localized ophthalmic infections, the plant pathogen *Fusarium oxysporum* routinely drives onychomycosis and invasive systemic fungal infections in immunosuppressed hosts (Nucci & Anaissie, 2002).

Certain plant pathogens belonging to the class Oomycetes, commonly found in aquatic agroecosystems and irrigation networks, possess highly aggressive infection pathways that cross the epithelial barriers of mammalian hosts, initiating intravascular dissemination and causing severe tissue ischemia. The capacity of these agents to induce vascular occlusion and sight-threatening keratitis, even in fully immunocompetent individuals, provides undeniable biosecurity evidence warranting prioritized monitoring under biosecurity standards. The proper adoption of standardized detection protocols for these aquatic pathogens is of paramount importance for safeguarding public health. Among the taxonomic groups capable of transitioning from botanical niches to mammalian systems, genera within the Oomycetes-most notably *Pythium*, *Lagenidium*, and *Aphanomyces*-pose the most critical risks (Cheung et al., 2026; Gaastra et al., 2010; Grooters, 2003). Once they breach the epithelial barrier, these organisms differentiate into invasive, thread-like hyphal structures that deeply penetrate subcutaneous tissues and vascular networks with a highly destructive tropism (Cheung et al., 2026; Gaastra et al., 2010; Grooters, 2003).

8. FOREST HEALTH AND PERENNIAL CROP PROTECTION WITHIN THE ONE HEALTH FRAMEWORK

In both forest and landscape ecosystems, introducing invasive forest pests that damage the vascular tissues of woody plants poses a profound risk of driving large-scale collapse across forest and crop production landscapes, primarily by vectoring incurable, lethal phytopathogenic bacteria.

Xylella fastidiosa, a devastating xylem-limited phytopathogenic bacterium primarily vectored by xylem-feeding insects such as the meadow spittlebug (*Philaenus spumarius*), has caused severe, multi-tiered economic and structural damage across global crop production regions (Bragard et al., 2019).

Historically localized to the Americas, this devastating pathogen was introduced to Southern Italy in 2013, triggering extensive desiccation across olive (*Olea europaea*) plantations (Ipsen, 2020). In the absence of effective therapeutic interventions, the infection systematically progresses to tree mortality (Sabelli, 2023). The resulting demise of approximately 21 million trees across 54,000 hectares in Italy, particularly within the monumental olive groves of Salento, represents an irreplaceable destruction of cultural heritage and vital ecosystem services (Ali et al., 2021). Following this biological invasion, local crop production dynamics were severely disrupted: the loss of vegetative cover increased soil temperatures, erosion, and evapotranspiration, while concomitantly reducing carbon sequestration and regional precipitation patterns, thereby elevating vulnerability to both floods and wildfires (Ali et al., 2021). Beyond direct impacts on woody crop production, these cascading environmental changes cause irreversible losses in forests, landscapes, and recreational spaces, ultimately undermining regional ecotourism (Ali et al., 2021).

Equipping future plant health and crop protection professionals with an integrated perspective on perennial ecosystems requires the successful execution of forestry and forest engineering concepts as flexible elective modules within existing agricultural curricula (Akan et al., 2024b). Transitioning these modules into standalone courses over the long term, as interdisciplinary collaborations expand, is of paramount importance to mitigate future deforestation, biodiversity loss, and systemic ecological collapse.

9. PESTICIDE TOXICOLOGY AND ECOLOGICAL HEALTH

The environmental migration of chemical pesticides used in crop production landscapes routinely results in uncontrolled drift into adjacent ecological niches. This off-target movement highlights how ecotoxicological hazards extend far beyond targeted pathogens, insect pests, or weeds. Instead, these residues disrupt soil microbiome function and cause detrimental effects on non-target biological communities, including natural enemies such as parasitoids and predators, pollinators, soil-dwelling macroorganisms, and aquatic taxa (Erdoğan et al., 2024).

Occupational exposure of agricultural workers during unregulated chemical applications in crop production fields, especially when personal protective equipment is neglected or label protocols are breached, triggers acute clinical poisoning while dramatically elevating chronic health hazards. Long-term contact is directly correlated with increased incidences of lung cancer, bladder cancer, and leukemia. Organophosphate exposure induces severe neuropathological disorders in the central nervous system, impairing critical cognitive functions such as learning and memory (Calliera et al., 2025; Erdoğan et al., 2024).

Transcending the narrow, target-centric limits of classical toxicology, the "One Toxicology" paradigm evaluates chemical risks across human, animal, and environmental compartments within a shared ecosystem. This holistic framework offers a next-generation methodology for assessing and mitigating cumulative pesticide pressures on planetary health. Synthesizing Beasley's joint toxicological assessment models with Rachel Carson's foundational ecotoxicological warnings provides a robust intellectual compass for guiding contemporary regulatory policies (Polat & Keçik, 2025).

Elucidating the adverse outcome pathways (AOPs) of pesticide toxicity at the cellular and molecular scales using innovative biotechnological methodologies provides a critical biosecurity mechanism for predicting organ-specific toxicities, such as hepatotoxicity, without relying on traditional mammalian assays. Integrating *in vitro* and *in silico* approaches, including high-throughput cell-culture assays, is of paramount importance to preemptively evaluate the adverse effects of novel active ingredients while addressing the ethical and logistical constraints of animal testing (Calliera et al., 2025).

While strobilurin-based fungal control agents exhibit exceptional efficacy in suppressing target phytopathogens, their potential to induce off-target adverse effects on cellular organelles in mammalian and aquatic organisms warrants rigorous monitoring. Because these fungal inhibitors drive significant mitochondrial toxicity in non-target animals, meticulous characterization of their toxicological profiles remains essential to prevent widespread, destructive ecological cascades (Yang et al., 2021).

10. INVASIVE SPECIES AND ECOSYSTEM RESILIENCE

Expanding global trade networks and anthropogenic climate shifts rapidly facilitate the introduction of invasive pests and phytopathogens into novel, historically naive ecological niches. Beyond inducing severe yield and quality losses within crop production systems, this geographic range expansion destabilizes food security across the "farm-to-fork" continuum and disrupts local ecological equilibria. The proper adoption of robust quarantine and biosecurity modules within agricultural higher education is of paramount importance to train students to comprehend these multidimensional disruptions, especially as uncontrolled alien biological invasions systematically dismantle ecosystem resilience, creating severe long-term biosecurity vulnerabilities (Lorenzini & Nali, 2025).

Habitat colonization by the emerald ash borer (*Agrilus planipennis*), an invasive, phloem-feeding beetle of Asian origin, illustrates how the loss of forest canopy and woody resources directly ripples through to public health crises. These infestations exacerbate the urban heat island effect and increase air-pollution-related respiratory and cardiovascular morbidity among human populations, compromising urban public health sustainability (Costilow et al., 2017; Martin, 2022). Over a decade of beetle spread across 15 states in the United States has been epidemiologically linked to more than 6,000 respiratory and 15,000 cardiovascular mortalities, exposing the raw physical links between urban forestry and human survival (Schrader et al., 2021).

The devastating ecosystem-level damage triggered by the soilborne oomycete *Phytophthora cinnamomi* provides indisputable evidence of how a single soil pathogen acts as a "biological bulldozer," completely decimating entire botanical populations and native biodiversity (Hardham & Blackman, 2018; Lowe et al., 2000; Richardson et al., 2000; Vear & Dell, 2001; Wills, 1993). Simultaneously, the xylem-limited quarantine bacterium *Xylella fastidiosa* drives cascading ecological disasters where its introduction not only decimates centuries-old olive plantations and invaluable cultural landscapes but also alters localized microclimates and accelerates soil degradation (Ali et al., 2021).

Safeguarding natural aquatic networks requires biosecurity policies to transcend terrestrial boundaries and integrate with freshwater ecology under a unified One Health framework. This transdisciplinary need is starkly

highlighted by *Aphanomyces astaci*, the causative oomycete agent of crayfish plague. Highly destructive and exotic to the Australian continent, this pathogen poses a catastrophic threat to endemic freshwater crayfish populations, necessitating its classification as a nationally notifiable disease to protect vulnerable aquatic trophic webs (Herbert, 2014).

11. NEMATODE CONTROL AND SOIL BIODIVERSITY

Managing phytoparasitic nematodes in crop production landscapes must move beyond the indiscriminate use of synthetic nematicides, shifting toward a holistic One Health paradigm that values the complexity of the soil food web. Preserving the ecological diversity of free-living, non-target nematodes and mutualistic soil microbiomes enhances the resilience of broader agroecosystems. Serving as primary bioindicators and key drivers of nutrient cycling, these beneficial free-living taxa sustain vital ecosystem functions, ultimately facilitating a strategic reduction in pesticide dependence (Semprucci et al., 2026; Tarasco et al., 2023).

Intensive synthetic nematicides used against plant-parasitic nematodes in conventional crop production cause persistent ecological degradation, prompting urgent research into the ecotoxicological safety of botanical volatile essential oils and other natural alternatives. Characterizing these bio-rational options is essential to safeguard beneficial soil organisms while minimizing public health hazards. Ecotoxicological evaluations of natural substitutes, such as the essential oils of basil (*Ocimum basilicum*), demonstrate that biopesticides can suppress target pests without disrupting non-target soil nematode communities, offering a viable pathway to reduce reliance on chemical inputs (Catani et al., 2025; Semprucci et al., 2026).

Integrating organic amendments such as raw compost and untreated animal manure is commonly practiced to improve soil structure and establish biological equilibrium. However, haphazard field applications in crop production can inadvertently introduce human pathogens, transforming horticultural soils into infection reservoirs. Fresh agricultural commodities, particularly leafy greens and root vegetables that are exposed to contaminated soil amendments or irrigation networks, can harbor persistent zoonotic pathogens, posing severe food safety hazards (CDC, 2025). Integrating stringent stabilization, pasteurization, and sanitation standards for organic

inputs is of paramount importance to prevent enteric pathogens from breaching biological barriers and triggering destructive foodborne outbreaks.

12. DIGITAL TECHNOLOGIES IN CROP PROTECTION

Integrating digital crop protection technologies into active crop production landscapes remains a fundamental prerequisite for establishing sustainable global food security and managing pre-harvest phytopathogens. Traditional survey and monitoring methodologies struggle to match the rapid, climate-driven progression of plant epidemics, making substantial yield losses inevitable without digital integration.

While applying chemical inputs protects global food supply networks, the off-target drift of pesticides from crop production fields into adjacent ecological niches continues to trigger unpredictable toxic synergies within biological populations and aquatic networks. The physical drift and macro-scale environmental fate of these plant protection products represent primary drivers of non-target wildlife exposure (Lonsdorf et al., 2024; Narvaez et al., 2022).

Addressing the complex dynamics of drift and contamination in digital crop protection curricula requires incorporating advanced frameworks, such as edge computing and neuro-fuzzy modeling architectures. These systems process microclimatic data locally at network nodes rather than relying on heavy cloud-server transmissions, thereby minimizing latency and optimizing energy consumption. An integrated computer model that combines wireless sensor networks with edge-computing technologies uses intelligent algorithms to analyze agronomic monitoring data to proactively predict crop diseases (Diachenko et al., 2025).

Incorporating smart crop production technologies into plant health modules not only equips future agronomists with critical digital competencies but also constructs a protective ecological shield for environmental and public health by optimizing pesticide application rates. The successful execution of this pedagogical model promotes efficient resource utilization, reduces dependence on chemical inputs through highly targeted interventions, and minimizes pathogen-induced yield losses, thereby driving agricultural sustainability (Diachenko et al., 2025).

Mitigating the off-target exposure and ecotoxicological risks associated with conventional chemical applications requires developing robust *in silico* environmental fate models designed to predict selective pressures on terrestrial and aquatic organisms, which is of paramount importance within the One Health framework. These predictive simulations evaluate how plant protection products behave across soil and aquatic matrices while simultaneously mapping complex organismal and human exposure pathways (Suciu et al., 2026).

13. BSc AWARENESS AND ACTION PLAN

Transforming undergraduate education in crop production and plant protection necessitates shifting students from passive recipients to self-directed, analytical professionals. This transition, guided by constructivist pedagogical frameworks, remains essential for implementing One Health principles in both open-field and protected crop production systems. Redesigning agricultural engineering curricula with a student-centered approach is of paramount importance to prepare graduates to develop proactive, integrated responses to complex ecological and clinical challenges (Akan et al., 2024a).

Integrating problem-based learning models, clinical case presentations, and laboratory simulations of contemporary plant health crises within the crop protection curriculum represents a dynamic educational methodology. This approach enables students to translate theoretical biological and phytopathological principles into real-world scenarios. Conducting face-to-face or online case analyses, with active participation by industry professionals, equips students with the analytical capacity to evaluate Integrated Pest Management (IPM) pathways through a multidisciplinary lens (Akan et al., 2024b).

To proactively manage biological and toxicological risks across the "farm-to-fork" value chain-particularly regarding enteric foodborne pathogens such as *Salmonella enterica*, *Listeria monocytogenes*, or *Escherichia coli*, and mycotoxins synthesized by *Aspergillus* and *Fusarium* species-students must autonomously coordinate self-designed crop production projects. This project-based active learning model instills professional competency, leadership, and systemic accountability, guiding students through all operational phases from seed selection to final consumer distribution to create resilient biosecurity protocols (Akan et al., 2024a).

Modern crop protection education must transcend the mechanical instruction of traditional diagnostic pathology and entomology, equipping students with the cognitive capacity to formulate rapid, innovative, and integrative solutions against non-routine global crises. These challenges include climate change, the dissemination of destructive invasive species, and the rise of antimicrobial resistance-such as azole resistance in *Aspergillus fumigatus*. This holistic pedagogical objective is a vital prerequisite for enabling future agronomists to meet shifting global market demands, develop critical thinking competencies, and solve complex, non-routine systemic challenges (Akan et al., 2024a).

To consolidate these disparate competencies into a unified academic identity, the phased modular framework must systematically converge into a dedicated, standalone cross-disciplinary training titled "Plant Health in One Health." This comprehensive course design bridges the operational gaps of fragmented learning by establishing explicit, multi-tiered learning outcomes. Under this integrated syllabus, students synthesize target-oriented phytoprotection protocols, pesticide ecotoxicology, vector transmission dynamics, and biosecurity regulations into a cohesive analytical framework.

Rather than evaluating biotic and abiotic risks in isolation, undergraduates engage in structured case studies and multidisciplinary problem-solving models that reflect real-world agricultural and public health emergencies. Establishing this dedicated course ensures that future plant protection professionals move beyond isolated diagnostic skills, acquiring the systemic capability to design, implement, and govern transdisciplinary biosecurity solutions across crop production ecosystems (Akan et al., 2024b; FAO, UNEP, WHO, & WOAH, 2022).

Ensuring the long-term sustainability of this educational action plan requires continuous "training of trainers" alongside the creation of robust mentorship, internship, and guidance networks that connect young researchers and recent graduates with the medical, veterinary, and environmental sectors. This structural coordination strengthens multisectoral workforce capacity, securing seamless interdisciplinary data and action integration. The proper adoption of structured placements, professional mentorship, and standardized competency frameworks is essential for incentivizing and supporting the next

generation of One Health practitioners, researchers, and technical officers (FAO, UNEP, WHO, & WOAH, 2022).

Enabling students to translate their theoretical One Health, plant health, and sustainable food safety competencies into active, field-level engagement represents a highly effective mechanism to foster societal awareness and drive behavioral modification, particularly among rural growers and youth. The effective implementation of these student-led extension activities and field workshops serves as a critical lever for localizing integrated plant health standards and food hygiene protocols, establishing a resilient crop production community.

CONCLUSION AND EVALUATION

Securing the contemporary sustainability of crop production systems against multi-tiered, escalating biotic and abiotic threats requires fundamentally redefining the theoretical position of plant health within the global biosecurity chain. Rather than treating phytoprotection as an isolated technical discipline, it is essential to ensure its seamless integration across all ecological and environmental compartments. Fortifying plant health as an indispensable primary shield against devastating ecological and macroeconomic shocks is of paramount importance to mitigate systemic global risks, yielding cascading benefits ranging from localized food security to macroeconomic stability.

Globally formulated strategic frameworks systematically unify human, animal, plant, and environmental health disciplines under a single, proactive objective: to build a preventive biosecurity shield against unforeseen pandemics and ecological crises, rather than relying on reactive, *post hoc* interventions. The anticipated impact of the “One Health Joint Plan of Action” lies in its capacity to create a world better equipped to prevent, predict, detect, and respond to systemic health threats, thereby driving sustainable development while systematically improving the collective health of humans, animals, plants, and their shared environments (FAO, UNEP, WHO, & WOAH, 2022).

Translating this holistic biosecurity vision into higher education requires the immediate, non-fragmented institutionalization of a dedicated standalone undergraduate course titled “Plant Health in One Health”. Bypassing piecemeal curricular modifications, the pedagogical trajectory of plant protection must center on an explicit 14-week analytical framework that systematically unites

phytopathology, ecotoxicology, food safety, and biosecurity governance into a coherent academic discipline (FAO, UNEP, WHO, & WOAH, 2022).

Dismantling traditional disciplinary boundaries through this phased educational roadmap equips future professionals with the systemic capability to mitigate complex planetary health risks. Mobilizing higher education curriculum developers, academic leadership, and environmental policymakers within a synchronized network represents the only viable pathway to embed plant health as a foundational pillar of global biosecurity (FAO, UNEP, WHO, & WOAH, 2022; OHHLEP, 2022).

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CHAPTER 7

MODEL SELECTION IN MOLECULAR PHYLOGENETICS: PRACTICAL INTERPRETATION OF AIC, AICc, AND BIC

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1. INTRODUCTION

The reconstruction of evolutionary relationships has long been one of the central objectives of biological research. Advances in molecular systematics have transformed phylogenetic inference from morphology-based analyses to computational approaches using nucleotide and genomic sequence data. Today, molecular phylogenetics plays a central role in plant systematics, evolutionary biology, conservation genetics, molecular ecology, comparative genomics, and biodiversity research, providing increasingly accurate insights into evolutionary history (Bos & Posada, 2005; Sullivan & Joyce, 2005; Kelchner & Thomas, 2007). The rapid development of high-throughput sequencing technologies has further accelerated this progress by enabling analyses based on hundreds of loci, complete organellar genomes, and even whole nuclear genomes. While these large datasets have improved phylogenetic resolution, they have also increased the need for analytical methods capable of accurately describing molecular evolution (Sullivan & Joyce, 2005; Xiang et al., 2023). DNA sequences do not directly reveal evolutionary relationships because nucleotide differences reflect complex evolutionary processes, including mutation, selection, genetic drift, recombination, and lineage-specific rate variation. Consequently, phylogenetic inference relies on nucleotide substitution models, which provide the statistical framework for likelihood- and Bayesian-based analyses by describing how substitutions accumulate through evolutionary time (Bos & Posada, 2005; Posada & Buckley, 2004). The overall workflow of substitution model selection in molecular phylogenetics is summarized in Figure 1.

As molecular datasets have become increasingly complex, selecting an appropriate substitution model has become a critical step in phylogenetic analysis. Different genes, genomes, and taxonomic groups exhibit distinct evolutionary patterns, and no single model adequately describes every dataset. An inappropriate model may bias parameter estimation, branch lengths, tree topology, and measures of phylogenetic support. Therefore, model selection should be regarded as an essential component of evolutionary inference rather than a routine computational procedure (Posada & Buckley, 2004; Susko & Roger, 2020; Xiang et al., 2023). Information-theoretic approaches such as the Akaike Information Criterion (AIC), the corrected Akaike Information

Criterion (AICc), and the Bayesian Information Criterion (BIC) are now widely used to compare competing substitution models by balancing model fit against complexity. Although these criteria are implemented in most phylogenetic software, their interpretation is often misunderstood, and recent studies continue to debate their influence on phylogenetic inference, particularly for heterogeneous and genome-scale datasets (Posada, 2008; Abadi et al., 2019; Susko & Roger, 2020; Liu et al., 2023).

This chapter provides a practical guide to model selection in molecular phylogenetics, with particular emphasis on the interpretation of AIC, AICc, and BIC. By combining theoretical concepts with examples from plant molecular phylogenetics, it aims to help graduate students and researchers make informed decisions when selecting and interpreting evolutionary models.

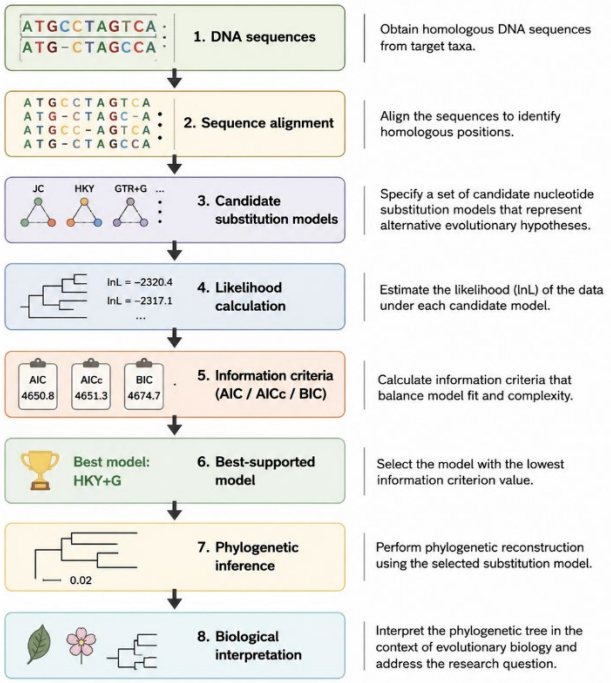

Figure 1. Overall workflow of model selection in molecular phylogenetics. General workflow illustrating the major steps of substitution model selection, from DNA sequence acquisition and sequence alignment to phylogenetic inference and biological interpretation.

2. FUNDAMENTALS OF EVOLUTIONARY MODELS

2.1 Why Do Evolutionary Models Matter?

The primary objective of molecular phylogenetics is to reconstruct the evolutionary history of organisms using homologous DNA or protein sequences. However, sequence similarity alone cannot accurately reflect evolutionary relationships because nucleotide differences result from complex processes such as multiple substitutions, unequal substitution frequencies, and lineage-specific evolutionary rates. Consequently, phylogenetic inference requires explicit evolutionary models that describe how nucleotide substitutions accumulate over time rather than relying solely on observed sequence differences (Bos & Posada, 2005; Sullivan & Joyce, 2005). Evolutionary models provide a mathematical framework for describing molecular evolution by incorporating biologically realistic features such as unequal nucleotide frequencies, transition–transversion bias, rate heterogeneity among sites, and invariant positions. These assumptions enable Maximum Likelihood and Bayesian methods to estimate phylogenetic trees, branch lengths, divergence times, and other evolutionary parameters within a statistically consistent framework (Kelchner & Thomas, 2007; Bos & Posada, 2005; Sullivan & Joyce, 2005).

Importantly, evolutionary models should not be regarded merely as computational options within phylogenetic software but as explicit biological hypotheses describing the substitution process underlying the observed sequence data. Because different datasets rarely evolve under identical conditions, no single substitution model is universally appropriate. Understanding the biological assumptions of evolutionary models is therefore essential for reliable model selection and robust phylogenetic inference (Bos & Posada, 2005; Sullivan & Joyce, 2005; Kelchner & Thomas, 2007).

2.2 What Is a Nucleotide Substitution Model?

A nucleotide substitution model is a mathematical representation of the evolutionary process by which one nucleotide is replaced by another over time. Rather than describing the full complexity of molecular evolution, substitution models provide simplified statistical approximations that capture the principal features of sequence evolution while remaining computationally tractable. Their primary purpose is to estimate the probability of nucleotide substitutions

along the branches of a phylogenetic tree, thereby enabling evolutionary relationships to be inferred under explicit biological assumptions (Bos & Posada, 2005; Sullivan & Joyce, 2005).

Substitution models differ in the evolutionary assumptions they incorporate, including nucleotide frequencies, substitution rates, transition–transversion bias, rate heterogeneity among sites, and the proportion of invariant positions. Because molecular datasets evolve under different evolutionary constraints, no single model adequately describes every dataset. Instead, models vary in the number and type of parameters used to approximate the underlying substitution process (Kelchner & Thomas, 2007; Bos & Posada, 2005). Importantly, substitution models are simplified representations of biological reality rather than exact descriptions of molecular evolution. The objective of model selection is therefore not to identify a "true" evolutionary model but to select the model that best approximates the evolutionary history represented by the available sequence data (Sullivan & Joyce, 2005; Kelchner & Thomas, 2007). This principle is particularly relevant in plant molecular phylogenetics, where different genomic regions often exhibit distinct evolutionary patterns. For example, ITS generally evolves more rapidly than many chloroplast coding regions, whereas plastome datasets frequently contain heterogeneous evolutionary signals. Understanding the assumptions underlying nucleotide substitution models therefore provides the conceptual foundation for selecting appropriate evolutionary models in phylogenetic analyses (Bos & Posada, 2005; Sullivan & Joyce, 2005).

2.3 Evolution of Nucleotide Substitution Models

2.3.1 Historical Development

The development of nucleotide substitution models has been driven by the need to describe molecular evolution with increasing biological realism while maintaining statistical tractability. Early models relied on simplified assumptions, whereas later models progressively incorporated biologically relevant features such as unequal nucleotide frequencies, transition–transversion bias, and rate heterogeneity. Consequently, the historical development of substitution models reflects a gradual increase in biological realism rather than the replacement of "incorrect" models by "correct" ones (Bos & Posada, 2005; Sullivan & Joyce, 2005)(Fig 2).

Table 1 summarizes the major nucleotide substitution models and highlights how each model introduced additional evolutionary parameters to overcome specific limitations of its predecessors. Together, these developments culminated in increasingly flexible models, such as GTR, which remain widely used in contemporary phylogenetic analyses (Bos & Posada, 2005; Sullivan & Joyce, 2005).

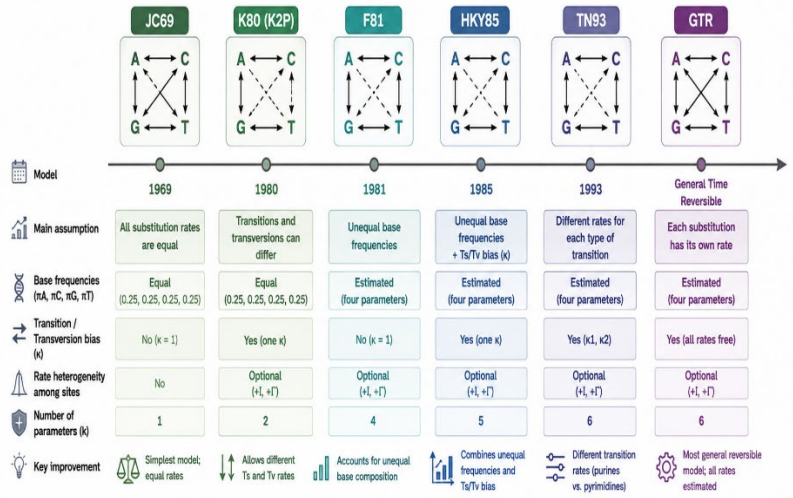

Figure 2. Historical evolution of nucleotide substitution models. Progressive development of nucleotide substitution models from JC69 to GTR, illustrating the gradual incorporation of biologically realistic assumptions.

Table 1. Historical progression of the major nucleotide substitution models, illustrating how increasingly realistic evolutionary assumptions have been incorporated into phylogenetic analyses over time.

Model	Introduced	Main assumptions	Improvement over previous models	Typical applications
JC69	1969	Equal base frequencies, equal substitution rates	First mathematical substitution model	Teaching, simple datasets
K80	1980	Transition ≠ transversion	Introduced Ts/Tv ratio	Closely related taxa
F81	1981	Unequal base frequencies	More realistic nucleotide composition	Moderate divergence
HKY85	1985	Unequal base frequencies + Ts/Tv bias	Combined K80 and F81 assumptions	Widely used in plant phylogenetics
TN93	1993	Different transition rates	Improved transition modeling	Diverse datasets
GTR	1986	All substitution rates estimated independently	Most parameter-rich reversible model	Complex phylogenetic datasets

2.4 Components of Modern Evolutionary Models

Modern nucleotide substitution models differ in the evolutionary parameters they incorporate to describe sequence evolution. Most models are based on a common set of biological assumptions, including nucleotide frequencies, substitution-rate variation among nucleotide pairs, transition–transversion bias, rate heterogeneity among sites, and the presence of invariant positions. Together, these components determine how accurately a model represents molecular evolution (Bos & Posada, 2005; Sullivan & Joyce, 2005). One of the most fundamental components is nucleotide composition. Early models assumed equal frequencies for the four nucleotides, whereas more advanced models estimate base frequencies directly from the observed sequence alignment. Because nucleotide composition often varies among genes, genomes, and taxonomic groups, allowing unequal base frequencies generally improves model fit. Another important feature is the distinction between transitions and transversions, as transitions typically occur more frequently than transversions. More complex models extend this approach by estimating separate substitution rates for all nucleotide pairs, providing greater flexibility for heterogeneous datasets (Bos & Posada, 2005; Kelchner & Thomas, 2007; Sullivan & Joyce, 2005).

Modern models also account for rate heterogeneity among sites because nucleotide positions rarely evolve at identical rates. This variation is commonly modeled using a gamma (Γ) distribution, while invariant sites are represented by the +I parameter. Although these additions often improve model fit, they also increase model complexity by introducing additional parameters that must be estimated during phylogenetic analysis (Posada & Buckley, 2004; Sullivan & Joyce, 2005). These evolutionary components explain why substitution models differ in both biological realism and statistical complexity. As models become more parameter-rich, they generally fit the observed data better but also become more susceptible to overparameterization. Balancing this trade-off between model fit and complexity forms the basis of information criteria such as AIC, AICc, and BIC, which are discussed in the following section (Bos & Posada, 2005; Posada & Buckley, 2004; Sullivan & Joyce, 2005).

3. LIKELIHOOD THEORY IN PHYLOGENETICS

3.1 Why Is Likelihood Needed in Phylogenetic Inference?

Although modern substitution models differ in both biological assumptions and statistical complexity, selecting the most appropriate model for a particular dataset remains a fundamental challenge in molecular phylogenetics. More complex models generally fit the observed sequence data better than simpler alternatives, making biological realism alone insufficient for model comparison. Consequently, phylogenetic inference requires an objective statistical framework capable of evaluating how well each evolutionary model explains the observed data while accounting for differences in model complexity (Sullivan & Joyce, 2005; Posada & Buckley, 2004). This need gave rise to likelihood-based phylogenetic inference, one of the most influential developments in molecular systematics. Rather than assessing whether a model appears biologically plausible, likelihood measures the probability of observing the available sequence data under a specified evolutionary model and phylogenetic tree. It therefore provides a quantitative basis for comparing competing evolutionary hypotheses within a common statistical framework (Bos & Posada, 2005; Sullivan & Joyce, 2005). However, likelihood alone is insufficient for model selection because models with more parameters generally achieve higher likelihood values simply due to their greater flexibility. If model selection relied exclusively on likelihood, the most parameter-rich model would almost always be preferred, regardless of whether the additional parameters reflect genuine evolutionary processes or merely random variation in the data. This phenomenon, known as overparameterization or overfitting, represents one of the central challenges in phylogenetic model selection and ultimately motivated the development of modern information criteria (Posada & Buckley, 2004; Sullivan & Joyce, 2005).

3.2 What Does Likelihood Measure?

Likelihood is one of the fundamental concepts underlying modern phylogenetic inference and forms the statistical foundation of both Maximum Likelihood (ML) and several model selection procedures used in molecular evolution. In its simplest interpretation, likelihood quantifies how well a particular evolutionary hypothesis explains the observed molecular sequence

data by evaluating the probability of obtaining the observed data under a specified evolutionary model, phylogenetic tree, and associated evolutionary parameters. Unlike descriptive measures of sequence similarity, likelihood therefore provides an objective statistical framework for comparing competing evolutionary hypotheses (Bos & Posada, 2005; Sullivan & Joyce, 2005). An important characteristic of likelihood is that it is always evaluated relative to a particular evolutionary model. Because different substitution models make different assumptions regarding nucleotide frequencies, substitution rates, transition–transversion bias, and rate heterogeneity among sites, the same molecular dataset may produce different likelihood values under different models. Thus, likelihood reflects the agreement between the observed sequence data and the assumptions of the selected evolutionary model rather than being an intrinsic property of the sequence alignment itself (Bos & Posada, 2005; Kelchner & Thomas, 2007). From a practical perspective, likelihood can be viewed as a measure of explanatory performance. Models with higher likelihood values generally provide a better statistical explanation of the observed sequence data. However, higher likelihood values are often achieved simply by increasing the number of estimated parameters. Therefore, likelihood alone cannot determine whether a more complex model offers a genuinely better biological explanation or merely greater mathematical flexibility. This limitation provides the rationale for penalized likelihood approaches such as AIC, AICc, and BIC, which are discussed in the following sections (Posada & Buckley, 2004; Sullivan & Joyce, 2005).

3.3 Why Do Phylogenetic Analyses Use Log-Likelihood Instead of Likelihood?

Although likelihood provides the statistical basis for evaluating competing evolutionary hypotheses, phylogenetic analyses almost always report the natural logarithm of likelihood (lnL) rather than raw likelihood values. This transformation does not change the biological interpretation of likelihood; instead, it simplifies calculations and improves numerical stability when analyzing large molecular datasets (Bos & Posada, 2005; Sullivan & Joyce, 2005). The need for log-likelihood arises because the likelihood of a sequence alignment is calculated by multiplying the probabilities of observing each nucleotide site. Since these probabilities are typically much smaller than

one, the resulting likelihood rapidly becomes an extremely small number that is difficult to represent accurately using standard computational precision. Converting likelihood values to their natural logarithms transforms these products into sums, making calculations more stable while preserving the relative ranking of competing evolutionary models (Bos & Posada, 2005; Sullivan & Joyce, 2005). An important consequence of this transformation is that log-likelihood values are usually negative because likelihood values range between zero and one. These negative values do not indicate poor model performance; rather, lnL is meaningful only when models are compared with one another. Models with higher likelihoods (i.e., less negative lnL values) provide a better statistical explanation of the observed sequence data under the assumptions of the corresponding substitution model (Posada & Buckley, 2004; Sullivan & Joyce, 2005). For this reason, lnL serves as the starting point for model selection rather than a final measure of model quality. Information criteria such as AIC, AICc, and BIC are all calculated directly from the log-likelihood while incorporating penalties for model complexity. Understanding the interpretation of lnL is therefore essential before applying any information criterion in substitution model selection (Posada & Buckley, 2004; Sullivan & Joyce, 2005).

3.4 The Number of Parameters and the Problem of Overparameterization

One of the fundamental characteristics of likelihood-based phylogenetic inference is that models with a larger number of free parameters almost always achieve higher likelihood values than simpler models when fitted to the same dataset. This is because additional parameters increase model flexibility, allowing more complex substitution patterns to be accommodated. However, a higher likelihood does not necessarily indicate a more accurate representation of biological reality, as it may simply reflect increased mathematical flexibility rather than genuine evolutionary signal (Sullivan & Joyce, 2005; Posada & Buckley, 2004). This phenomenon, known as overparameterization, occurs when increasingly complex models begin to fit random variation in addition to the true evolutionary signal. Although such models often produce higher likelihood values, they may reduce the reliability of parameter estimates and the generalizability of phylogenetic inference, particularly for datasets with

limited informative sites or taxon sampling (Bos & Posada, 2005; Sullivan & Joyce, 2005).

Conversely, overly simple models may fail to account for important evolutionary processes such as unequal nucleotide frequencies, transition–transversion bias, or rate heterogeneity among sites, leading to systematic bias. Therefore, phylogenetic model selection aims to identify the model that provides the best balance between goodness-of-fit and model complexity rather than simply selecting the simplest or most complex alternative (Kelchner & Thomas, 2007; Posada & Buckley, 2004; Sullivan & Joyce, 2005). Balancing model fit and complexity therefore requires an appropriate statistical framework. Information criteria such as the Akaike Information Criterion (AIC), the corrected Akaike Information Criterion (AICc), and the Bayesian Information Criterion (BIC) evaluate improvements in model fit while simultaneously penalizing unnecessary complexity, providing a statistically justified framework for substitution model selection (Posada & Buckley, 2004; Sullivan & Joyce, 2005).

4. INFORMATION CRITERIA FOR MODEL SELECTION

4.1 Why Were Information Criteria Developed?

Information criteria were developed to address one of the fundamental challenges in statistical model selection: balancing model fit against model complexity. In molecular phylogenetics, increasingly complex substitution models almost always achieve higher likelihood values because additional parameters provide greater flexibility for describing the observed sequence data. However, improved statistical fit does not necessarily indicate a more accurate representation of the underlying evolutionary process, while overly simple models may fail to capture important features of molecular evolution. Consequently, model selection requires an objective framework that evaluates both explanatory performance and model complexity rather than relying on likelihood alone (Sullivan & Joyce, 2005; Posada & Buckley, 2004). Information criteria provide this balance by combining model fit with a penalty for the number of estimated parameters (Fig 3). As a result, more complex models are preferred only when the improvement in likelihood is sufficient to justify the additional complexity. Rather than identifying the model with the highest likelihood, these approaches aim to select the model that achieves the

most appropriate compromise between explanatory power and statistical simplicity, consistent with the principle of parsimony that underlies much of biological inference (Johnson & Omland, 2004; Bos & Posada, 2005; Sullivan & Joyce, 2005). Among the various approaches proposed, three criteria have become the most widely used in molecular phylogenetics: the Akaike Information Criterion (AIC), the corrected Akaike Information Criterion (AICc), and the Bayesian Information Criterion (BIC). Although all three combine likelihood with a penalty for model complexity, they differ in their theoretical foundations and practical interpretation. Understanding these differences is essential for selecting an appropriate substitution model and interpreting model selection results in phylogenetic analyses (Posada & Buckley, 2004; Susko & Roger, 2020; Sullivan & Joyce, 2005).

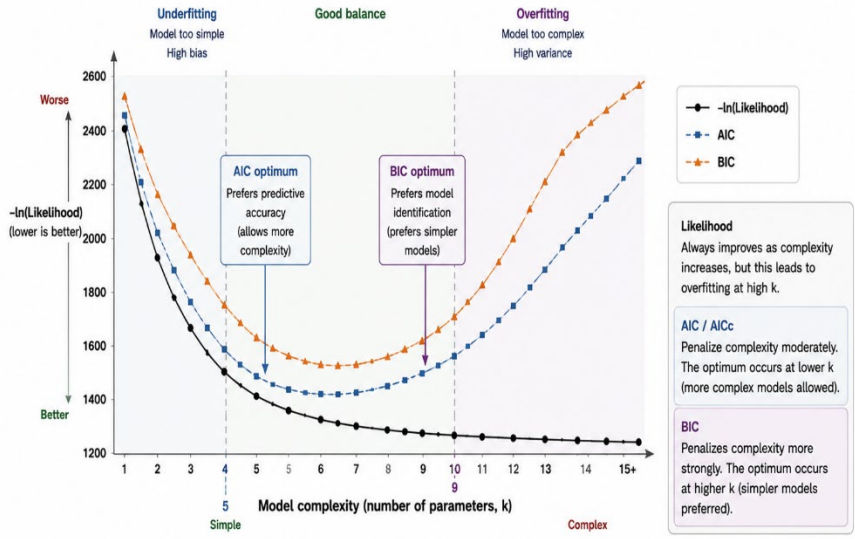

Figure 3. Relationship between likelihood and model complexity. Likelihood improves as model complexity increases, whereas information criteria identify an optimal balance by penalizing unnecessary parameters.

4.2 The Akaike Information Criterion (AIC)

4.2.1 Concept and Mathematical Formulation

The Akaike Information Criterion (AIC) is one of the most widely used information-theoretic approaches for substitution model selection in molecular phylogenetics. Rather than identifying the model with the highest likelihood

alone, AIC evaluates whether improvements in model fit are sufficient to justify the additional complexity introduced by estimating more parameters. Consequently, AIC seeks to identify the model expected to provide the best balance between explanatory performance and model complexity while minimizing unnecessary overparameterization (Johnson & Omland, 2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005). Unlike traditional hypothesis-testing approaches, AIC assumes that competing substitution models represent alternative approximations of the evolutionary process rather than true or false descriptions of biological reality. The objective is therefore not to identify the "true" evolutionary model, but to select the model expected to lose the least amount of information when explaining the observed molecular sequence data. This information-theoretic perspective has made AIC one of the most influential model selection criteria in evolutionary biology and molecular phylogenetics (Johnson & Omland, 2004; Sullivan & Joyce, 2005).

AIC combines model fit and model complexity into a single criterion:

$$AIC = -2\ln(L) + 2k$$

where lnL is the log-likelihood of the fitted model and k is the number of estimated parameters. The likelihood term rewards models that explain the observed data more successfully, whereas the penalty term discourages unnecessary increases in model complexity. Consequently, a more complex substitution model is preferred only when the improvement in likelihood is sufficient to offset the additional penalty associated with estimating extra parameters. Thus, AIC represents a direct extension of likelihood theory by integrating model fit and complexity into a single model selection criterion (Johnson & Omland, 2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005).

4.2.2 Interpretation of AIC

AIC is a relative model selection criterion, meaning that its numerical values are meaningful only when comparing alternative substitution models fitted to the same molecular dataset. The preferred model is the one with the lowest AIC value, indicating the best compromise between explanatory performance and model complexity among the candidate models evaluated. Consequently, AIC values have no absolute biological meaning and should not be interpreted as indicators of overall analytical quality (Johnson & Omland,

2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005)(Table 2). For example, the General Time Reversible (GTR) model may produce a higher likelihood than simpler models such as HKY or TN93 because it estimates more parameters. However, if the improvement in likelihood is relatively small, the complexity penalty imposed by AIC may outweigh this gain, resulting in the selection of a simpler substitution model. Conversely, when the increase in likelihood is sufficiently large, AIC may favor the more complex model. Therefore, AIC rewards statistically justified complexity rather than complexity itself (Akaike 1973; Posada & Buckley, 2004; Bos & Posada, 2005).

Model selection should not rely solely on identifying the model with the lowest AIC value. Instead, researchers should also evaluate the magnitude of the differences among competing models using ΔAIC (Delta AIC), calculated as the difference between the AIC value of each candidate model and that of the best-supported model. The best model has ΔAIC = 0, whereas increasing ΔAIC values indicate progressively weaker empirical support. Rather than serving as a statistical significance test, ΔAIC provides a practical measure of the relative evidence supporting competing evolutionary models (Johnson & Omland, 2004; Sullivan & Joyce, 2005).

Table 2. General interpretation of ΔAIC values in phylogenetic model selection.

ΔAIC	Interpretation	Practical implication
0–2	Substantial support	Competing models are similarly supported; biological interpretation should also be considered.
4–7	Considerably less support	The best model is generally preferred, although alternative models cannot be completely dismissed.
>10	Essentially no support	The competing model is unlikely to represent the observed data as effectively as the best-supported model.

Although ΔAIC indicates the relative support for competing models, it does not quantify how strongly each model is supported within the candidate set. For this purpose, Akaike weights (wAIC) estimate the proportion of evidence supporting each model relative to all competing models. Values range from 0 to 1, with higher values indicating stronger relative support. However,

Akaike weights should not be interpreted as the probability that a model is objectively true; rather, they express the relative likelihood that a model provides the best approximation of the evolutionary process among the models evaluated (Johnson & Omland, 2004; Posada & Buckley, 2004). Together, ΔAIC and Akaike weights provide a more informative interpretation than model ranking alone. While ΔAIC measures the relative difference between candidate models, Akaike weights describe how the available evidence is distributed across the entire model set, helping researchers avoid overconfidence when several substitution models receive similar levels of statistical support (Table 3) (Johnson & Omland, 2004; Sullivan & Joyce, 2005).

Table 3. Example interpretation of Akaike weights.

Model	AIC	ΔAIC	Akaike weight	Interpretation
HKY+G	2456.2	0.0	0.58	Highest relative support
GTR+G	2457.1	0.9	0.37	Strong competing model
TN93+G	2461.8	5.6	0.05	Limited support

4.3 Corrected Akaike Information Criterion (AICc)

4.3.1 Concept and Mathematical Formulation

The Corrected Akaike Information Criterion (AICc) was developed to overcome an important limitation of the original Akaike Information Criterion when applied to relatively small datasets. Although AIC performs well when sample size is large relative to the number of estimated parameters, it may favor unnecessarily complex substitution models when available data are limited because the penalty for model complexity becomes insufficient (Johnson & Omland, 2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005). This limitation is particularly relevant in molecular phylogenetics, where analyses often rely on relatively short molecular markers such as ITS, matK, or rbcL. In such datasets, the number of informative nucleotide sites may be limited relative to the complexity of candidate substitution models, increasing the risk of overparameterization. To address this problem, AICc incorporates an

additional correction term that increases the penalty for model complexity when sample size is small, while retaining the information-theoretic framework of the original AIC (Sugiura 1978; Hurvich & Tsai 1989; Johnson & Omland, 2004; Posada & Buckley, 2004).

The criterion is expressed as:

$$AICc = AIC + \frac{2k(k+1)}{n-k-1}$$

where AIC is the Akaike Information Criterion, k is the number of estimated parameters, and n is the sample size. As the sample size increases, the correction term approaches zero and AICc converges to AIC. Conversely, when sample size is limited relative to model complexity, the correction becomes increasingly important, reducing the tendency to select unnecessarily complex substitution models (Johnson & Omland, 2004; Sullivan & Joyce, 2005).

4.3.2 Interpretation and Practical Use

The interpretation of AICc closely parallels that of AIC. Candidate substitution models are ranked according to their AICc values, with the model exhibiting the lowest value considered to provide the most appropriate balance between model fit and complexity under finite-sample conditions. Like AIC, AICc values have no absolute biological meaning and should only be compared among models fitted to the same molecular dataset (Johnson & Omland, 2004; Posada & Buckley, 2004). The principal advantage of AICc is that it provides a more conservative evaluation of model complexity when available data are limited. As a result, simpler substitution models may be preferred unless additional parameters produce a sufficiently large improvement in likelihood. For large datasets, the difference between AIC and AICc becomes negligible because the correction term approaches zero. Consequently, AICc should be regarded not as a replacement for AIC but as its small-sample extension, making it particularly appropriate for analyses based on short molecular markers or datasets with limited phylogenetic information (Johnson & Omland, 2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005).

4.4. Bayesian Information Criterion (BIC)

4.4.1 Concept, Mathematical Formulation and Interpretation

The Bayesian Information Criterion (BIC) provides an alternative approach to model selection that places greater emphasis on identifying the simplest model adequately supported by the available data. Unlike AIC, which was developed primarily to optimize predictive performance, BIC is based on Bayesian model comparison and applies a stronger penalty for increasing model complexity. Consequently, BIC generally favors simpler substitution models unless additional parameters produce substantial improvements in model fit (Johnson & Omland, 2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005). This stronger penalty becomes particularly important for large molecular datasets. Whereas AIC applies a constant penalty for each additional parameter, BIC increases the penalty as sample size grows, reflecting the principle that stronger evidence should be required before accepting more complex evolutionary models. As a result, BIC often selects less parameter-rich substitution models than AIC, especially in analyses involving extensive sequence alignments or phylogenomic datasets (Schwarz 1978; Johnson & Omland, 2004; Sullivan & Joyce, 2005).

The Bayesian Information Criterion is expressed as:

$$BIC = -2\ln(L) + k\ln(n)$$

where lnL is the log-likelihood, k is the number of estimated parameters, and n is the sample size. Similar to AIC, the first term evaluates model fit, whereas the second penalizes model complexity. However, because the penalty increases with sample size, increasingly strong statistical evidence is required to justify additional parameters as datasets become larger (Johnson & Omland, 2004; Posada & Buckley, 2004).

Like AIC and AICc, BIC is a relative model selection criterion and should only be used to compare models fitted to the same molecular dataset. Importantly, differences between AIC and BIC should not be interpreted as evidence that one criterion is universally superior. Rather, they reflect different statistical objectives: AIC emphasizes predictive performance, whereas BIC places greater emphasis on model parsimony and identification. Consequently,

the choice between these criteria should depend on the characteristics of the dataset and the objectives of phylogenetic study rather than on a preference for a single information criterion (Table 4) (Johnson & Omland, 2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005).

Table 4. Philosophical comparison of AIC, AICc and BIC.

Feature	AIC	AICc	BIC
Primary objective	Predictive accuracy	Predictive accuracy with finite-sample correction	Identification of the most strongly supported model
Statistical philosophy	Information theory	Information theory	Bayesian approximation
Complexity penalty	Constant	Constant + small-sample correction	Increases with sample size
Effect on complex models	Moderate	Moderate to strong	Strong
Behaviour with small datasets	May overfit	Recommended	Often conservative
Behaviour with large datasets	Stable	Converges to AIC	Increasingly conservative
Practical emphasis	Prediction-oriented model comparison	Particularly useful when sample size is limited relative to model complexity	Model identification with a stronger complexity penalty

5. PRACTICAL RECOMMENDATIONS FOR MODEL SELECTION IN PLANT MOLECULAR PHYLOGENETIC STUDIES

5.1 From Statistical Results to Biological Interpretation

The ultimate objective of substitution model selection is not simply to identify the model with the lowest information criterion but to improve the biological interpretation of phylogenetic analyses. Although modern software automatically calculates likelihood values, information criteria, and model rankings, these outputs should be regarded as decision-support tools rather than definitive answers. Reliable model selection requires the integration of statistical evidence with biological knowledge and evolutionary reasoning (Sullivan & Joyce, 2005; Posada & Buckley, 2004). In plant molecular phylogenetics, the characteristics of the molecular marker should always be considered alongside the selected substitution model. Nuclear markers such as

ITS generally exhibit higher substitution rates and greater sequence variability than many chloroplast coding regions, whereas plastome datasets often contain heterogeneous evolutionary patterns among coding and non-coding regions. Consequently, information criteria should be interpreted within the biological context of the molecular marker, taxonomic group, and research objectives rather than applied as universal computational rules (Bos & Posada, 2005; Kelchner & Thomas, 2007; Sullivan & Joyce, 2005).

Disagreement among AIC, AICc, and BIC should not be regarded as evidence of analytical error. Instead, these criteria reflect different statistical philosophies and may legitimately recommend different substitution models for the same dataset. In such cases, reporting the results obtained from multiple criteria together with a biological justification for the selected model provides greater transparency and improves the reproducibility of phylogenetic analyses (Johnson & Omland, 2004; Susko & Roger, 2020; Posada & Buckley, 2004). Ultimately, substitution model selection represents one component of the broader phylogenetic inference process(Fig 4). Robust evolutionary conclusions depend not on selecting the most complex model but on choosing a statistically appropriate model that is biologically meaningful. Phylogenetic software assists model selection, but scientific interpretation remains the responsibility of the researcher (Burnham & Anderson 2004; Sullivan & Joyce, 2005; Bos & Posada, 2005).

6. PRACTICAL INTERPRETATION OF MODEL SELECTION RESULTS

6.1 Interpreting Model Selection Results

Model selection should be regarded as the transition from statistical estimation to biological interpretation rather than as a purely computational procedure. Although phylogenetic software automatically identifies the best-fitting substitution model, researchers should interpret the results in the context of the molecular marker, dataset characteristics, and biological objectives of the study (Sullivan & Joyce, 2005; Posada & Buckley, 2004). The selected substitution model represents the best-supported approximation among the candidate models evaluated rather than the true model of nucleotide evolution (Table 5). Consequently, statistical evidence should always be considered

together with biological knowledge when making phylogenetic inferences (Burnham & Anderson 2002; Johnson & Omland, 2004; Bos & Posada, 2005).

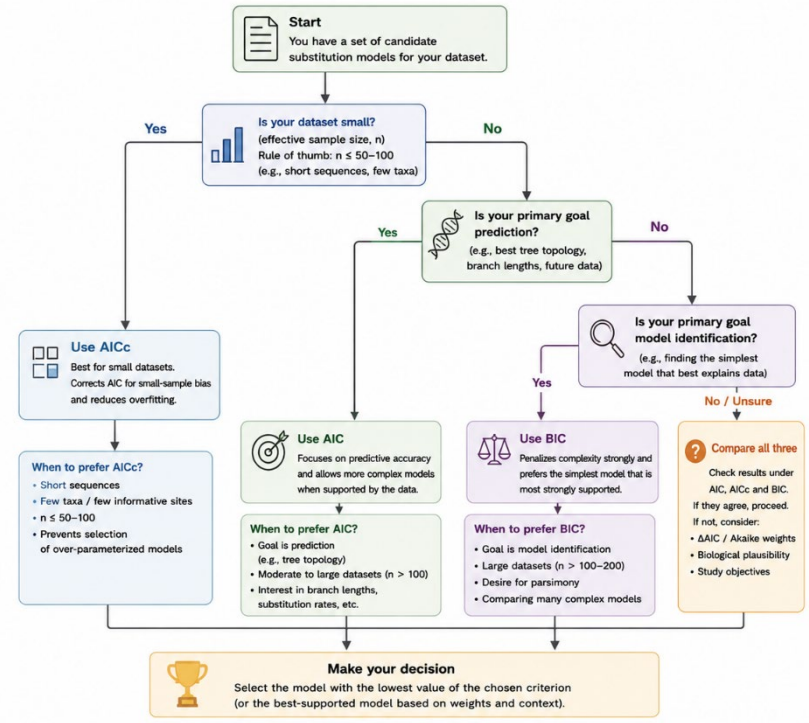

Figure 4. Decision tree for selecting an appropriate information criterion. Simplified guide for choosing AIC, AICc, or BIC according to dataset characteristics and analytical objectives.

Table 5 illustrates a simplified example of the output generated by model-selection software.

Model	-lnL	Parameters (k)	AIC	AICc	BIC	Interpretation
JC	2456.8	1	4915.6	4915.6	4921.4	Poor fit despite simplicity
HKY+G	2320.4	5	4650.8	4651.3	4674.7	Best compromise between fit and complexity
GTR+G	2317.1	9	4652.2	4656.5	4693.8	Highest likelihood but greater complexity

The numerical values presented in this table are hypothetical and are provided solely to demonstrate the interpretation of information criteria.

6.2 Interpreting a Typical Model Selection Output

At first glance, the GTR+G model exhibits the highest likelihood because it explains the observed sequence variation more effectively than the simpler alternatives. However, the additional parameters required by GTR+G increase the penalty imposed by both AIC and BIC. Consequently, HKY+G becomes the preferred model because it provides a better balance between model fit and model complexity. This example illustrates one of the central principles discussed throughout this chapter: the model with the highest likelihood is not necessarily the model selected by information criteria (Johnson & Omland, 2004; Posada & Buckley, 2004).

6.3 How Should Model Selection Be Reported in Scientific Articles?

Transparent reporting of substitution model selection is essential for ensuring the reproducibility of phylogenetic analyses. Unfortunately, many published studies report only the name of the selected substitution model without describing the criterion used for model selection or the software employed. Such incomplete reporting limits the reproducibility of phylogenetic analyses and prevents readers from evaluating the robustness of the analytical procedure.

The following examples illustrate good and poor reporting practices.

- Example of inadequate reporting: “The HKY+G model was selected for phylogenetic analysis. The statement above provides no information regarding the statistical criterion, the software used, or the analytical framework under which the model was selected”.
- Example of recommended reporting: “The best-fitting nucleotide substitution model was determined using the Bayesian Information Criterion (BIC) implemented in MEGA11. Among the candidate models evaluated, HKY+G exhibited the lowest BIC value and was subsequently used for maximum-likelihood phylogenetic reconstruction.”

This reporting style clearly communicates the criterion applied, the software used, and the rationale for selecting the substitution model, thereby improving the transparency and reproducibility of the study (Sullivan & Joyce, 2005; Posada & Buckley, 2004).

6.4 Common Mistakes in Model Selection

Despite the widespread availability of automated model-selection software, several mistakes remain common in molecular phylogenetic studies. Most of these errors arise from misunderstanding the interpretation of information criteria rather than from the statistical methods themselves (Table 6).

Table 6. Frequent mistakes encountered during substitution model selection

Common mistake	Why is it problematic?	Recommended practice
Selecting the model with the highest likelihood	Ignores model complexity	Evaluate AIC, AICc or BIC together with likelihood
Reporting only the selected model	Reduces reproducibility	Report the software, criterion and selected model
Comparing AIC values between different datasets	AIC values are dataset-specific	Compare only candidate models from the same analysis
Ignoring ΔAIC or Akaike weights	Overstates confidence in a single model	Evaluate the relative support for competing models
Using the same substitution model for every marker	Evolutionary processes differ among markers	Perform model selection separately for each dataset

7. CURRENT CHALLENGES AND FUTURE PERSPECTIVES

7.1 Is Model Selection Always Necessary?

Substitution model selection has long been considered a fundamental step in molecular phylogenetic analyses and is routinely implemented in software such as MEGA, IQ-TREE, and ModelFinder (Posada & Buckley, 2004; Sullivan & Joyce, 2005). However, recent studies have questioned whether exhaustive model selection is always necessary. Based on simulation analyses, Abadi et al. (2019) suggested that parameter-rich substitution models often produce phylogenetic trees similar to those obtained after formal model selection, particularly when tree topology is the primary objective. Despite this

debate, the importance of model selection depends on the biological objectives and characteristics of the dataset. While tree topology may be relatively robust to model choice, estimates of branch lengths, substitution rates, and divergence times can be more sensitive (Susko & Roger, 2020). For plant molecular phylogenetic studies based on relatively short markers such as ITS, matK, rbcL, and trnL, appropriate model selection remains an important step for ensuring analytical reliability and methodological transparency.

7.2. Emerging Trends in Model Selection

Advances in high-throughput sequencing have shifted molecular phylogenetics from single-gene analyses toward plastome, transcriptome, and genome-scale datasets. As data complexity increases, applying a single substitution model to an entire alignment is often insufficient to represent heterogeneous evolutionary processes. Modern software therefore incorporates automated model-selection algorithms and partition-specific approaches that improve computational efficiency while accommodating evolutionary heterogeneity. Programs such as IQ-TREE integrate ModelFinder, which enables the efficient evaluation and selection of substitution models within modern phylogenetic workflows (Kalyaanamoorthy et al., 2017).

CONCLUSION

Substitution model selection is a fundamental component of molecular phylogenetics because it provides an objective framework for evaluating competing evolutionary hypotheses while balancing model fit and complexity. As discussed throughout this chapter, model selection extends beyond the automatic recommendations generated by phylogenetic software and requires the integration of statistical evidence with biological knowledge to support reliable phylogenetic inference (Johnson & Omland, 2004; Posada & Buckley, 2004; Sullivan & Joyce, 2005). AIC, AICc, and BIC represent complementary rather than competing approaches to model selection. Each criterion reflects a different statistical objective and may therefore recommend different substitution models for the same dataset. Consequently, selecting an appropriate model depends not only on statistical performance but also on the characteristics of the molecular dataset and the biological objectives of the study (Johnson & Omland, 2004; Susko & Roger, 2020).

Recent developments in phylogenomics, automated model-selection algorithms, and emerging computational approaches continue to refine how evolutionary models are evaluated. Nevertheless, these advances reinforce rather than replace the need to understand the assumptions underlying model selection and to interpret statistical results within their biological context (Abadi et al., 2019; Susko & Roger, 2020). Ultimately, model selection is not the search for the most complex model or simply the model with the lowest information criterion. Instead, it is the process of identifying the evolutionary model that provides the most biologically meaningful and statistically justified explanation of the observed molecular data. Phylogenetic software can recommend candidate models, but informed scientific judgment remains the responsibility of the researcher.

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CHAPTER 8

HEAVY METAL STRESS RESPONSES IN THE ASTERACEAE FAMILY: PHYSIOLOGICAL, BIOCHEMICAL, AND MOLECULAR MECHANISMS OF TOLERANCE

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1. INTRODUCTION

Heavy metal pollution upon increasing industrialization, mining activities and intensive agricultural practices has become a serious environmental problem affecting both terrestrial and aquatic ecosystems. Amount of toxic heavy metals is increasing day by day because of the anthropogenic effects and the accumulation of heavy metals in soil and water ecosystems is accelerating (Jarin et al., 2025). Heavy metals are nondegradable unlike many organic pollutants, and they can be persistent in the environment where they can be taken up by plants and can enter food web. Effects of heavy metals on plants largely depend on the concentration in the environment and the chemical form; while elements such as iron (Fe), zinc (Zn), copper (Cu) and nickel (Ni) have a role as micronutrients for plants in low amounts, no known physiological function of elements like cadmium (Cd), lead (Pb), and mercury (Hg) and they can show toxic effects even in small amounts (Sevgi & Leblebici, 2022).

Excessive accumulation of heavy metals in plants can disrupt many physiological and cellular processes; seed germination, growth of roots and seedlings, photosynthesis and water and nutrient uptake can be impaired. In cellular level, metal toxicity can impair membrane integrity, functions of chloroplast and mitochondria, and metabolic homeostasis. Moreover, heavy metals cause the formation of reactive oxygen species (ROS) and can lead to oxidative damage in lipids, proteins and nucleic acids. The intensity of the effects depends on the type of metal, concentration, exposure duration, plant genotype and environmental conditions (Jorjani & Karakaş, 2024).

Plants have developed complex physiological, biochemical and molecular defense mechanisms to overcome the toxicity upon heavy metals. In order to limit the toxicity, plants develop various defense mechanisms such as regulating metal uptake and mobility, immobilizing metal ions in the cell wall, chelating with phytochelatin and metallothionein, storing metals in vacuoles, and enhancing enzymatic and non-enzymatic antioxidant defense mechanisms (Jarin et al., 2025). The antioxidant defense system, which includes enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, and glutathione reductase, plays a central role in controlling metal induced oxidative stress. In addition, changes in primary and secondary metabolisms, stress signalization, transcriptional regulations and metal carriers form the metal tolerance (Gill &

Tuteja, 2010).

Asteraceae family represents an important plant group for investigation of effects of heavy metals on plants. Asteraceae is one of the largest and widely distributed angiosperm families which have the ability to colonize several habitats including heavy metal contaminated environments. The importance of Asteraceae is also coming from the metal tolerance, hyperaccumulating metals, and from the detailed physiological, biochemical and molecular research on these processes. Genera such as *Helianthus, Senecio, Berkheya, Centaurea, Tagetes* and *Taraxacum* belonging to Asteraceae were reported in research about heavy metal stress (Bech et al., 2012; Jorjani & Karakaş, 2024; Ojuederie & Babalola, 2017). However, current information has been distributed among different heavy metal types, experimental systems and biological stress levels. Comparative evaluation of physiological, biochemical and molecular responses of species of Asteraceae family is important to understand tolerance mechanisms. Therefore, this study aims to summarize the heavy metal responses of Asteraceae family. Heavy metal uptake and accumulation, physiological changes, oxidative stress and antioxidant defense, metabolic regulations, molecular mechanisms of tolerance and phytoremediation potential are especially emphasized.

2. Asteraceae Family: Diversity and Ecological Importance

Asteraceae is one of the important plant families in natural ecosystems and agricultural systems owing to the species richness, large geographical distribution, adaptation capacity to various environmental conditions. This family shows a distribution from tropical to subpolar ecosystems, from grasslands to arid lands to metal contaminated lands. Adaptation strategies against environmental stress elements by their morphological and physiological diversity made the members of Asteraceae family model group for plant ecology, evolutionary biology and environmental sciences. Some genera have high tolerance to heavy metals and the ability to accumulate them make the highlights this family for phytoremediation studies. In this chapter, taxonomic and ecological diversity of Asteraceae family together with their importance in heavy metal stress studies will be taken into account.

2.1. Ecological Diversity of Asteraceae Family and Adaptation to Environmental Stress

Asteraceae family is one of the families with largest and the most ecologically diverse families in angiosperms. There are around 1600-1700 genera and 24.000-30.000 species according to the latest taxonomical studies. This family shows a distribution on every continent except Antarctica and has a large geographical distribution from tropical regions to high altitudes (Fu et al., 2016). Members of Asteraceae can grow in deserts, forests, grasslands, wetlands, alpines and saline habitats (Palazzesi et al., 2022).

This large ecological distribution of the family shows the high adaptation capacity of the members to temperature changes, water scarcity, different soil characteristics and other environmental stress conditions. The presence of annual and perennial herbaceous species, as well as shrubs, vines, and, rarely, tree like species, makes the Asteraceae family an important model group for studying the adaptation and tolerance mechanisms that evolve in response to environmental stresses (Nikolić & Stevović, 2015; Petrova et al., 2024). In this context, the ability of certain species to thrive in metal rich or contaminated soils is particularly noteworthy in terms of studying tolerance mechanisms against heavy metal stress.

2.2. Asteraceae Species in Metal-Contaminated Environments

Metal rich habitats such as mining areas, ultramafic and serpentine soils, industrial and urban areas form strong selective pressure on plants. High concentrations of potentially toxic metals such as Ni, Cd, Pb, Zn, Cu, Cr, and As can negatively affect plant growth, mineral nutrient balance, photosynthetic activity and cellular metabolism. On the contrary, some plants can survive under such conditions by the help of physiological, biochemical and molecular mechanisms which regulate metal uptake and intercellular distribution of metals.

There are many species in Asteraceae family that can adapt to metal rich environments or accumulate metals in their tissues. For example, *Berkheya coddii* is accepted as an important Ni hyperaccumulator; some species of *Senecio* and *Centaurea* genera have been reported to adapt ultramafic andNi rich soils (Robinson et al., 2003; Siebert et al., 2018). *Helianthus annuus*, which has the capacity to produce high biomass, is widely used in phyto-extraction

studies due to its accumulation of metals such as Cd, Pb and Zn (Angelova et al., 2016).

Metal tolerance in Asteraceae species is provided by co-functioning of mechanisms like limiting metal uptake, regulation of carrying metals from roots to shoot, binding of metal ions to cell wall, chelation with phytochelatin and metallothionines, and sequestration in vacuoles (Nikolić & Stevović, 2015). Comparative investigation of these mechanisms in different species contributes to understanding of physiological, biochemical and molecular basis of heavy metal tolerance and hyperaccumulation. Moreover, this knowledge forms the scientific basis of determination of the appropriate species for environmental scanning and phytoremediation applications for the rehabilitation of metal contaminated areas.

3. Heavy Metal Uptake, Transport, and Accumulation

Heavy metal accumulation in plants is a process with many phases determined by the bioavailability of metals in rhizosphere, transport in plants, and sequestration in different tissues. Soil pH, organic matter content, root exudates, microorganisms, and physiological characteristics of the plants play role in this process besides the chemical properties of the metals. Metal uptake and accumulation can vary among the Asteraceae species, and the differences can determine the hyperaccumulation capacity as well as the metal tolerance.

3.1. Heavy Metal Availability in the Rhizosphere

Uptake of metals by plants is related more to the kind of the metal, its mobility, and bioavailability in rhizosphere more than its concentration in soil. Metals can be dissolved, adsorbed, complexed, or precipitated, and the balance between these phases determines the amount of the metal to be taken up by the plants. Diffusion in rhizosphere, mass flow, contact with roots, soil pH, organic matter, and root exudates regulate the metal mobility (Seshadri et al., 2015).

pH is of the main determinant of heavy metal bioavailability. In general, low pH increases the solubility and mobility of many metals, high pH promotes adsorption, precipitation, and complex forming and hence decreases the dissolved metal fraction. However, this effect may change upon kind of metal, soil mineralogy and organic matter content (Mohamed et al., 2025). In a study on *Taraxacum officinale* heavy metals Cd, Cr, Cu, Fe, Pb, and Zn were shown

to accumulate in a high level in roots and shoots of the plant growing on mining wastes, and metal level in soil was shown to be strongly related with metal concentration in plant tissues. Researchers reported that low pH and low cation exchange capacity increased the metal solubility and mobility (Bini et al., 2012).

Soil organic matter can both immobilize or mobilize the metals. Organic matter can bind the metals via adsorption and forming complexes; while low molecular weight organic acids such as citrate, malate and oxalate which are secreted from the roots can increase metal solubility and mobility (Nedjimi, 2021). Root exudates may also affect metal uptake by changing pH of the rhizosphere; but the direction of this effect depends on the plant, kind of metal and soil conditions (Podar & Maathuis, 2022).

Rhizosphere microorganisms are important regulators of metal bioavailability. Bacteria and fungi can change the metal movement by biosorption, precipitation, redox reaction and formation of complexes. Especially plant growth promoter rhizobacteria and arbuscular mycorrhizae can change the response to heavy metal stress by varying root development and metal transport (Gupta et al., 2024). A study on *Helianthus annuus*, inoculation with metal tolerant plant growth promoter rhizobacteria strains *Ralstonia eutropha* and *Chrysiobacterium humi* showed that these strains increased the plant biomass and changed the metal accumulation in soil contaminated with Cd and Zn. Especially, *C. humi* significantly changed Cd and Zn accumulation in roots and shoots, and it revealed the role of rhizosphere microorganisms in metal bioavailability and distribution of metals inside the plants (Marques et al., 2013). The direction of this effect can change upon plant and microbial community structure, kind of metal and soil characteristics (Mishra et al., 2017).

As a result, heavy metal accumulation in Asteraceae species is evaluated not only by the physiological and genetic background of the plant, but also physicochemical and microbial characteristics of the rhizosphere. After the reaching of the bioavailable metals to root surface and uptake into the cell, transport in the plant and cellular detoxification processes determine the tolerance and accumulation of the heavy metals.

3.2. Root Uptake and Cellular Entry of Heavy Metals

Entrance of heavy metals to the plant body starts with the uptake of metal ions reaching to the root surface from the rhizosphere by root cells. Since roots are the basic contact surface between soil and plants, they are the main region for metal uptake. However, metal accumulation in roots does not solely occur through passive diffusion; ionic affinities, electrochemical gradients, membrane transporters, and mechanisms regulating metal homeostasis all play a role.

Metal uptake by roots and retention of metals show significant differences between Asteraceae species. For instance, *Helianthus annuus* stands out with phytoextraction of Cd, Pb and Zn heavy metals thanks to its strong root system and high biomass production (Ojuederie & Babalola, 2017). *Taraxacum officinale* and *Artemisia* species are frequently used in biomonitoring and phytoremediation studies because of their metal accumulation and tolerance characteristics (Bini et al., 2012; Mishra et al., 2017). The differences are related to the interspecies differences in root morphology, root surface area, rhizosphere interactions, and membrane transport systems.

The first significant adsorption region of metal ions reaching to root surface is the cell wall. Pectin, hemicellulose, and other negatively charged polysaccharides bind the cationic metals and contribute to their retention in apoplast and limitation of their transfer to cytoplasm. Heavy metals with low mobility, such as Pb, can be retained in high concentrations in root tissues. Studies on *H. annuus* showed that significant amounts of Cd and Pb accumulate in roots and transfer from roots to shoots change upon kind of metal, plant variety and environmental conditions (Rivelli et al., 2012).

Heavy metal ions crossing the cell wall are transferred to cytoplasm by transport proteins on the plasma membrane. Since completely specific transport systems are limited for heavy metals in plants, ions like Cd^{2+} and Ni^{2+}make use of proteins having role in essential elements of similar ionic characteristics. For instance, Cd^{2+} is related with Zn^{2+} and Ca^{2+} transport systems; Ni^{2+} and Fe^{2+} are related with Mg^{2+} and Zn^{2+} transporting systems. Because of this common carrying pathways, heavy metal stress can harm mineral nutrient balance in plants (Clemens, 2006).

Metals entering roots are carried to root tissues by apolaustic or

symplastic paths. Apoplastic movement occur along the spaces between cell walls and cells, Casparian strip in endodermis requires the selective uptake of metal ions into the cell. In symplastic movement, after ions are transported to the neighboring cells via cytoplasm and plasmodesma after they pass the plasma membrane. This selective transport makes it possible to regulate the loading of metals to xylem and transport of them from roots to shoots (Lux et al., 2011).

Since free ionic forms of intracellular metals can cause toxicity, plants detoxify them by complexing them with amino acids, phytochelatin, organic acids, and metallothionines. Significant amount of metal complexes is sequestered in vacuoles and removed from the cytoplasm (Angulo-Bejarano et al., 2021). Therefore, metal tolerance is not only related with limitation of metal uptake, but also with intracellular binding and compartmentalization mechanisms.

Experimental findings on Asteraceae species show that metal uptake is not always directly related to tolerance. Thanks to the strong root system and high biomass of H. annuus, it is effective for removal of especially Pb and Cd from contaminated soil and holds promise for phytoremediation (Alaboudi et al., 2018). *T. officinale* is used for biomonitoring since it can widely grow in metal contaminated urban habitats. Studies on populations showed that distribution of Pb, Ni, Mn and Zn in root, shoot and leaves change upon metal type, environmental conditions and habitat characteristics (Vural, 2024). Similarly, research on *Artemisia fragrans* showed that heavy metal tolerance can not only be defined with high metal uptake; selective distribution among different tissues, intracellular compartmentalization and protection of photosynthetic tissues also play important role in tolerance (Alirzayeva et al., 2017).

As a result, heavy metal uptake in Asteraceae species is a coordinated process of cooperation of adhesion on root surface, entrance into cell over membrane, apoplastic and symplastic movement and intracellular complex forming and sequestration. That is why not only total metal concentration but also retention in roots, transfer from roots to shoots and distribution of metals among organs should be taken into account for the evaluation of metal tolerance and phytoremediation potential.

3.3. Intracellular and Long-Distance Transport of Heavy Metals

Transport of heavy metals uptaken into the root cells to other tissues and intercellular distribution is regulated with coordinated activity of various membrane transport proteins.. In this process ZIP (ZRT/IRT-like Protein), NRAMP (Natural Resistance-Associated Macrophage Protein), HMA (Heavy Metal ATPase), MTP/CDF (Metal Tolerance Protein/Cation Diffusion Facilitator) and ABC (ATP-Binding Cassette) carrier families play role (Angulo-Bejarano et al., 2021). Among these carriers ZIP and NRAMP proteins regulate the metal uptake of bivalent metal ions like Zn^{2+}, Fe^{2+}, Mn^{2+} and Cd^{2+} into cells and transport among tissues, while transporters belonging to HMA play critical roles in transporting metals ions to xylem, carrying outside the cell and sustaining cellular metal balance. On the other hand, MTP/CDF and ABC transporters carry metal ions or metal-chelate complexes to the cellular parts like vacuoles and decrease free metal concentration in cytoplasm and limit metal toxicity (Verbruggen et al., 2009).

After the regulated metal homeostasis in root cells, long range transport of heavy metals happens via xylem and phloem. When heavy metals are loaded into xylem in the roots, metals are transported to shoot and leaves by transpiration flow, some elements are redistributed to young tissues, developing organs and reproduction structures via phloem. Moreover, mobility of metal ions in plants largely depends on the type of metal, its chemical form, complexes with ligands and activity of the related transporter proteins (Clemens et al., 2002).

The mobility of heavy metals from roots to above ground parts depends on chemical properties of the metal and physiological characteristics of the plant in Asteraceae family, too. *Helianthus annuus* is a species widely used in phytoremediation studies because of its high biomass and rapid development. Studies on contaminated soils with Zn and Pb, *H. annuus* was shown to uptake both metals, while Zn was more efficiently transported to shoots better than Pb, Pb was shown to accumulate more in root tissues (Adesodun et al., 2010).

Controlled transport of heavy metals in plant bodies and balance distribution among different tissues contribute both to decrease heavy metal toxicity and sustain the physiological balance of essential micronutrient elements. Intracellular chelating, vacuolar sequestration and selective transport by transporter proteins work together and try to inhibit the toxic heavy metals to damage the metabolic processes. That is why the gene expression of transporter proteins, distribution of them according to the tissue are among the main mechanisms in Asteracease family determining the hyperaccumulation capacity, heavy metal tolerance and phytoremediation potential (Hanikenne & Nouet, 2011).

4. Physiological Responses

Heavy metals can affect basic physiological processes like root development, water and nutrient uptake, photosynthesis, transpiration, growth and biomass production. The severity of these effects can change upon type and concentration of the metal, exposure duration, developmental stage of the plant and species-specific tolerance mechanisms. Members of Asteraceae family have developed adaptations like retaining metals in the roots, limiting roots to shoot transfer, maintenance water balance, and sustaining photosynthetic activity. On the other hand, heavy metals reaching toxic concentrations can cause numerous adverse physiological effects, such as suppression of root and shoot growth, reduced biomass accumulation, decreased leaf area, disruption of water relations, and weakened photosynthetic capacity (Hall, 2002).

4.1. Changes in Root Development and Biomass Production

Since roots are the first structures for direct heavy metal contact, early symptoms of metal toxicity occur in roots. Heavy metal exposure can decrease root growth, lateral root formation, root hair development, and total root biomass. These changes are related to suppression of cell division and elongation, disruption of nutrient uptake, changes in cell wall structure, and increased oxidative stress (Shahid et al., 2017).

Suppression of root development decreases the capacity of roots to uptake water and nutrient elements and indirectly affects shoot development. As a result, reduction in leaf area, slow shoot development, and decrease in total biomass occur. In species with high heavy metal tolerance, maintenance of root

development in a certain extent and increasing retaining in root cells are among the important physiological adaptations contributing to the protection of upper structures from metal toxicity. The extent of these physiological responses changes upon kind of metal, exposure duration, and species-specific tolerance mechanisms.

Studies on *Helianthus annuus* revealed that Cd and Pb stress negatively affected root elongation and total biomass, however, retaining of metal ions largely in root tissues decrease metal concentration transferred to shoot and supported heavy metal tolerance (Alaboudi et al., 2018). Similarly, in ecotypes of *Artemisia fragrans*, retaining of metal ions in roots and safe storage in intracellular compartments are evaluated among the main physiological adaptation mechanisms limiting toxic effects (Alirzayeva et al., 2017).

4.2. Water Relations and Transpiration

Heavy metal stress can significantly affect water relations and water use efficiency in plants. Damaged root system decreased root hydraulic conductivity and decreased water uptake efficiency upon metal toxicity negatively affect the water balance of the plant. In addition, heavy metals can cause the secretion of abscisic acid which stimulate the closure of stoma. Although closure of stoma is a protective mechanism to limit excessive water loss, it also reduces CO_2 uptake and hence limits carbon assimilation (Poschenrieder et al., 2006).

Transpiration flow not only plays a role in protecting water balance of the cell, but also in the transfer of metal ions from roots to above ground parts. Therefore, changes in transpiration rate can directly affect the distribution of heavy metals through xylem. Transpiration flow can increase metal translocation of especially heavy metals like Zn and Cd which have high mobility, but this effect can be limited for metals with lower mobility, like Pb (Verbruggen et al., 2009).

Leaf water potential, relative water content and stomatal conductivity were shown to decrease in Asteraceae species. However, these effects were shown to be limited in the high tolerant species. For example, Cd and Ni stresses in *Helianthus annuus* were shown to significantly decrease leaf water potential, relative water content, stomatal conductivity and transpiration rate which affected photosynthetic performance. In high metal tolerant species, it

was shown that disruption in water relations was limited and this was shown to contribute to metal tolerance (Ahmad et al., 2023). This reveals that conservation of water relations is one of the physiological adaptations in Asteraceae family heavy metal tolerance.

4.3. Changes in Photosynthetic Performance and Chlorophyll Content

One of the most significant physiological disturbances caused by heavy metal stress is reduction in photosynthetic performance. Heavy metals can damage in different steps of photosynthesis by suppressing chlorophyll biosynthesis, disrupting chloroplast ultrastructure and adversely affecting photosystem function. As a result, decrease in chlorophyll a and b contents, reduction in electron transfer rate and carbon assimilation can be observed (Gill & Tuteja, 2010).

Studies on the species from Asteraceae family showed that heavy metal stress adversely affected photosynthetic capacity. *Helianthus annuus* shoots of one week were placed into Hoagland solution containing 0.05, 0.07 and 0.09 mM $CdCl_2$ and grown for 10 days. Cadmium application decreased total chlorophyll content by 19.2%, 26.3% and 31.6% in order of dose, respectively. The findings revealed that the mentioned decrease was due to the suppression of chlorophyll biosynthesis of cadmium and limited photosynthetic capacity (Zengin & Munzuroglu, 2006).

The severity of this effect changes upon type of metal, exposure duration and plant specific tolerance mechanisms. In species with high metal tolerance loss of chlorophyll is limited, structural integrity of photosynthetic systems is better preserved and that photosynthetic activity can be maintained to a certain extent. This situation shows that preservation of photosynthetic system is an important physiological indicator of heavy metal tolerance in Asteraceae species.

4.4. Alterations in Mineral Nutrient Uptake and Homeostasis

Heavy metal stress can significantly affect essential nutrient element uptake, transfer and distribution among the tissues in plants. One of the major reasons of this situation is that heavy metals such as Zn^{2+}, Fe^{2+}, Ca^{2+}, $Mg^{2+,}$ and Mn^{2+} possess similar physiochemical characteristics with essential nutrient

elements and carried with the same membrane transporters. That is why exposure to heavy metals can form competition between toxic metals and essential elements and can damage mineral nutrient balance (Clemens et al., 2002).

In a study on *Helianthus annuus* were exposed to 0–1000 µM Cd. Upon increased cadmium concentrations, Zn, K and Ca Uptake significantly decreased in shoots, while Cd accumulation in plant tissues continuously increased. Significant decrease was observed in developmental tolerance index, relative water contents and total chlorophyll contents in the highest Cd dose, total accumulation of mineral nutrient elements decreased. The findings of the research revealed that the mentioned effects were because of the competition of cadmium ions with essential cations for usage of common transporters and as a result mineral homeostasis was disturbed (Çıkılı et al., 2020).

Heavy metals not only decrease nutrient element uptake but also change the expressions of carrier proteins such as ZIP, NRAMP, and HMA and affect the mineral element distribution in the plants. As a result, insufficiencies of elements like Fe, Zn, Ca, Mg, and Mn occur. This situation can cause various physiological disturbances such as enzyme activities, photosynthesis, respiration, and other metabolic processes (Hall, 2002).

In a study on *Helianthus annuus* applications of Cd, Zn and Cu significantly affected essential nutrient element homeostasis. As a result of heavy metal applications Changes in the levels of Fe, Mg, K, and Ca were observed, especially in the combined applications of Cd, Zn, and Cu, around 15% decrease was reported in total biomass and in leaf area. Researchers reported that the competition between essential elements and toxic metals disturbed mineral nutrition balance and this adversely affected plant growth and physiological parameters (Rivelli et al., 2012).

The preservation of nutrient elements homeostasis is one of the major physiological characteristics determining mental tolerance in Asteraceae species upon heavy metal stress. Moreover, in species with higher tolerance against heavy metals, selective functionality of carrier proteins, regulated transfer of metal ions, functions of essential elements in metabolic processes are conserved to a certain extent. On the contrary, in species with lower tolerance, disruption of mineral nutrition balance results in decrease of photosynthetic performance Ants growth retardation.

4.5. Effects on Growth and Development

Heavy metal stress is one of the important abiotic stress factors that affect general growth and development of plants. Metal toxicity suppresses cell division and cell elongation, photosynthetic carbon assimilation, and disturbs energy metabolism, and hence limits the growth potential of plants. As a result of these processes, developments of shoot and roots slow down, the leaf area reduces, biomass production and general developments performance decrease. As the severity and exposure of heavy metal stress increases, growth retardation becomes more Distinct, early signs of premature ageing may be observed, along with a decline in reproductive development and seed yield (Rascio & Navari-Izzo, 2011).

Studies conducted on Asteraceae family support this trend. When 0.05–0.09 mM Cd was applied to *Helianthus annuus* shoots, it was established that it significantly inhibits shoot and root development. In the same study, the reduction in total chlorophyll content by 19.2–31.6 % and in total soluble protein content by 22.5–45 %, depending on the applied Cd dose, suggests that the growth retardation is linked to the adverse effects on photosynthetic pigments and metabolic processes. Moreover, increase of proline and abscisic acid levels suggests that although growth was suppressed, physiological adaptation mechanisms against stress were activated.

However, species in the Asteraceae family do not exhibit similar growth responses to heavy metal stress. Metal ions binding to cell walls, being stored in vacuoles and efficient metal homeostasis in hyperaccumulators and high tolerant species, growth and biomass production can be preserved up to a certain extent. On the contrary, disruption of mineral nutrition balance, decrease of photosynthetic capacity and increase of oxidative damage in low tolerant species cause suppression of root and shoot development, decrease of leaf area and loss of biomass (Clemens, 2006; Hall, 2002; Rascio & Navari-Izzo, 2011). These findings demonstrate that the maintenance of growth and development is one of the key physiological indicators for assessing heavy metal tolerance in Asteraceae species.

5. Oxidative Stress and Antioxidant Defense

Heavy metal stress causes overproduction of reactive oxygen species and so oxidative stress. Reactive oxygen species act as signal molecules at low levels; but in the case of disruption of the balance between production and

detoxification, oxidative damage can occur on lipids proteins nucleic acids and cellular membranes. That is why plants developed a multi-phase defense system composed of enzymatic and nonenzymatic antioxidants in order to limit oxidative stress caused by heavy metals. In this session ROS formation mechanisms and antioxidant defense systems' main components will be discusses (Gill & Tuteja, 2010; Sharma et al., 2012).

5.1. Generation of Reactive Oxygen Species under Heavy Metal Stress

Heavy metal stress is one of the main environmental stress factors causing oxidative stress in plants. Even though some heavy metals like Cd, Hg, and Pb do not directly take place in redox reactions, they caused over expression of reactive oxygen species by disrupting electron transfer chain and suppressing antioxidant defense system (Gill & Tuteja, 2010). On the other hand redox active metals like Cu, Fe, and Cr take part in Fenton and Haber–Weiss reactions and directly promote production of high-level reactive oxygen species like hydroxyl (Sharma & Dietz, 2009).

Under heavy metal stress reactive oxygen species, mainly superoxide radical, hydrogen peroxide, hydroxyl radical and singlet oxygen are produced in plants. While these molecules play in cell signaling at low levels, in the case of productions take over antioxidants defense mechanism oxidative stress occurs. The formed ROS adversely affect cellular functions by damaging lipids, proteins, nucleic acids and photosynthetic pigments (Apel & Hirt, 2004; Mittler, 2017).

The main sources of reactive oxygen species are chloroplasts mitochondria peroxisomes and NADPH oxidases placed on plasma membrane. During heavy metal exposure the production of reactive oxygen species increase due to disruption of photosynthetic electron transfer in chloroplasts respiration electron transfer in mitochondria. Moreover, controlled ROS production through NADPH oxidases on plasma membranes, play an important role in transfer of stress signals in the cell and activation of defense mechanisms (Mittler, 2017).

ROS production increases upon heavy metal exposure in the members of Asteraceae family and this situation is one of the main oxidative stress responses in plants. According to a study on Cd tolerant and Cd sensitive

varieties of *Helianthus annuus,* increasing Cd concentrations (0–100 μM) increased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in leaves. In contrast, activities of superoxide dismutase and peroxidase which are important components of antioxidant defense system, were found to be higher especially in tolerant genotypes and this was observed to limit oxidative stress more efficiently. In addition, expression levels of metal carrier genes belonging to ZIP and NRAMP families were shown to change upon heavy metal stress; it was shown that this change is particularly effective in regulating the intracellular transport, accumulation and tolerance mechanisms of cadmium. The findings show that ROS production and antioxidant defense mechanisms are complementary and closely related processes in heavy metal stress response (Fu et al., 2022).

5.2. Oxidative Damage and Degeneration of Cellular Components

Disruption of the balance between reactive oxygen species production and their scavenging by antioxidant defense system causes oxidative damage in plant cells. Overproduction of ROS under heavy metal stress since they can cause disruption of cellular structures and functions by reacting with unsaturated fatty acids in cell membrane, proteins, nucleic acids, photosynthetic pigments. The severity of oxidative stress can change upon type of metal, exposure duration and concentration of the metal (Gill & Tuteja, 2010; Hasanuzzaman et al., 2020).

Cell membranes are one of the main targets of ROS. Hydroxyl radical especially starts lipid peroxidation by oxidizing multiple non saturated fatty acids found in membrane phospholipids. This process causes decrease membrane fluidity, increasing membrane permeability and ion leakage. Increase in the malondialdehyde levels which is the most common biochemical indicator of lipid peroxidation, is used in evaluation of membrane damage caused by heavy metal stress (Apel & Hirt, 2004).

ROS not only affect membrane lipids, but also proteins and nucleic acids. They cause oxidation of amino acid side chains, the disruption of their three-dimensional structure and reduction of biological activities of enzymes. Similarly, hydroxyl radical causes changes in DNA bases, strand breaks and mutations. These changes adversely affect cellular metabolism and causes

suppression of growth and development (Sharma & Dietz, 2009).

In a study different cadmium concentration (0, 20, 60 and 100 µM) effects on oxidative damage in *Artemisia annua.* While decrease in chlorophyll and carotenoids contents are observed with cadmium applications, electrolyte leakage, which is an indicator of disruption of cell membrane integrity, was shown to increase. In addition, malondialdehyde content which is accepted as an indicator of lipid peroxidation was shown to increase especially at the beginning phases of cadmium applications, and this situation was evaluated as the oxidative damage of metal. Moreover, changes in the levels of ascorbic acid and glutathione were emphasized to be components of antioxidant defense mechanisms to balance oxidative stress caused by heavy metal and to decrease cellular damage (Li et al., 2012). These findings demonstrate that the disruption of cell membrane integrity and lipid peroxidation under heavy metal stress are among the key indicators of oxidative damage in Asteraceae species.

5.3. Enzymatic Antioxidant Defense System

While increased ROS production upon heavy metal stress cause oxidative damage in plants, enzymatic defense system composing of enzymes such as superoxide dismutase, catalase, ascorbate peroxidase, peroxidase, glutathione reductase and glutathione peroxidase, play a major role in ROS homeostasis (Gill & Tuteja, 2010). In this system superoxide dismutase turns superoxide radical into H_2O_2, which is the first step of the defense, the formed H_2O_2 is turned into water by catalase and ascorbate peroxidase. The other three enzymes contribute to maintaining this antioxidant cycle and cellular redox balance (Hasanuzzaman et al., 2020).

Stress response of antioxidant enzymes also changes upon the kind of metal, exposure duration, plant species and tissue type. Low to medium level metal stress usually stimulates antioxidant enzyme activities, while high concentration or longer exposure can damage enzymatic system and decrease activities (Mansoor et al., 2023). Therefore, changes in the activities of these enzymes are among main biochemical indicators of heavy metal tolerance in plants. One of the important examples of this response in Asteraceae family was shown on *Tagetes erecta*. Applications of Cd (50–300 mg kg^{-1}) and Cu (150–400 mg kg^{-1}) cased a decrease in biomass, leaf number, and photosynthetic pigments, and an increase in lipid peroxidation and significant

activity levels of the above-mentioned enzymes. These results showed that coordinated activation of antioxidant enzymes limited heavy metal-based ROS accumulation and contributed the heavy metal tolerance and phytoremediation potential of *T. erecta* (Goswami & Das, 2017).

In conclusion, the enzymatic antioxidant system plays a central role as a defense mechanism in regulating ROS, limiting oxidative damage and maintaining cellular homeostasis under heavy metal stress. In particular, the coordinated and controlled activation of antioxidant enzymes is considered to be one of the key indicators of heavy metal tolerance in Asteraceae species.

5.4. Nonenzymatic Antioxidants

Besides enzymatic antioxidant system, in order to limit the oxidative damage caused by heavy metals a nonenzymatic defense system consisting of ascorbic acid (AsA), glutathione (GSH), tocopherols, carotenoids, phenolic compounds and flavonoids. These compounds directly neutralize ROS and contribute to protecting cellular redox balance (Sharma et al., 2012). Especially AsA and GSH play a significant role in removing as being main components of ascorbate-glutathione cycle H_2O_2 and maintaining the activity of antioxidant enzymes such as APX and GR. Moreover, while phenolic compounds and flavonoids scavenge ROS and reduce the reactivity of metal ions by chelating them, carotenoids and tocopherols contribute to the removal of ROS formed particularly in photosynthetic tissues and to the preservation of chloroplast structure (Gill & Tuteja, 2010; Michalak, 2006).

The role of these metabolites in stress response in Asteraceae species depends on the kind of metal, concentration, exposure duration and developmental stage of the plant. A study on *Carlina acaulis* reported that Cd application changed AsA, GSH and phytochelatin levels, increased the concentration of phenolic compounds, especially chlorogenic acid. These metabolites contributed to both scavenging ROS and chelating Cd and limit oxidative damage (Dresler et al., 2020).

Non enzymatic antioxidants are important adaptive mechanisms which support not only ROS detoxification but also metal chelation, redox homeostasis and protection of membrane and photosynthetic systems in Asteraceae family. Therefore, changes in AsA, GSH and phenolic metabolites can be used as important indicators for evaluation of biochemical tolerance

against heavy metal stress.

6. Biochemical and Metabolic Responses

Heavy metal stress not only changes physiological processes and oxidative balance, but also primary and secondary metabolic pathways. Plants generate various biochemical and metabolic responses to decrease metal concentration and reactivity in cell, protect cellular homeostasis and sustain basic metabolic processes. Among these responses are synthesizing metal binding compounds, regulating organic acid metabolism and changing phenolic compounds and flavonoids. The quality and intensity of these metabolic responses can vary upon plant species, chemical characteristics and concentration of the metal, and exposure duration (Clemens, 2006).

6.1. Phytochelatins and Metallothioneins

One of the main detoxification mechanisms in case of heavy metal is the metal binding molecules which are rich in cysteine. Main components of this system are phytochelatin and metallothionein, and they can bind heavy metals like Cd, Pb, Hg, Cu, Zn, and As via their high cysteine content. This way, they decrease free metal concentration in cytoplasm and contribute to protecting cellular structures from metal toxicity (Cobbett & Goldsbrough, 2002).

Phytochelatin is cysteine rich peptide, which is not directly encoded by genes, but synthesized from reduced glutathione via phytochelatin synthase (PCS). Especially PCS activated by Cd starts rapid phytochelatin synthesis, the formed phytochelatin-metal complex is carried to vacuole by ATP-dependent ABCC carrier proteins and removed from active cytoplasm (Seregin & Kozhevnikova, 2023). On the other hand, metallothionein is cysteine rich, low molecular weight proteins encoded by genes. Besides binging metal ions, it plays a role in Cu and Zn homeostasis, redox balance and control of oxidative stress (Cobbett & Goldsbrough, 2002; Ioniță et al., 2026). That is why phytochelatin and metallothionein are accepted as defense mechanisms which complement via metal chelation, intracellular metal homeostasis and limit oxidative stress (Hasan et al., 2017).

Experimental evidence obtained from Asteraceae members supports the importance of this mechanism. Application of 15–50 μM $Cd(NO_3)_2$ on *Helianthus annuus* significantly increased phytochelatin synthesis; Cd levels in

roots in the 9th day of 50 µM Cd application was reported to be 49.84 µmol g^{-1} fresh weight, and around 30 µmol g^{-1} fresh weight in leaves: the levels in roots were 1.3-1.8 times higher than the leaves (Yurekli & Kucukbay, 2003). Similarly, 0–100 µM Cd application on *Carthamus tinctorius* increased phytochelatin and non-protein thiol levels in roots, which was around 3 times of that of control. GSH level in leaves increased with increasing Cd level up to 50 µM Cd but decreased by higher Cd levels upon being used in phytochelatin production (Namdjoyan et al., 2012).

These findings indicate that GSH–phytokelatin metabolism and metal-binding proteins in Asteraceae species make a significant contribution to heavy metal tolerance, particularly under Cd stress, through the chelation of metal ions, vacuolar sequestration, and the mitigation of oxidative damage.

6.2. Proline and Other Osmoprotectant Metabolites

Heavy metal stress results in osmotic imbalance and increased ROS production in plants. In order to limit these effects of heavy metal stress, plants accumulate osmoprotective metabolites such as proline, glycine, trehalose, saccharose and other soluble sugars. These molecules help to protect osmotic homeostasis, maintain protein and membrane stability, protect enzyme activities and decrease oxidative damage (Zulfiqar et al., 2020).

Proline is one of the amino acids accumulating in case of osmotic regulation during heavy metal stress and has various roles protein and membrane stabilization, ROS limitation and redox homeostasis protection. Moreover, it forms complex with some metal ions and contributes to growth after stress and decreasing metal toxicity (Hayat et al., 2012). Glycine betaine especially supports chloroplast and photosynthetic structures, while trehalose, saccharose and other soluble carbohydrates contribute to maintaining metabolic functions under stress conditions by sustaining osmotic balance and membrane integrity (Ashraf & Foolad, 2007).

Experimental evidence of this response was shown on *Helianthus annuus*. Zengin and Munzuroğlu (2006) reported that leaf proline levels increased in *H. annuus* shoots exposed to 0.05, 0.07 and 0.09 mM $CdCl_2$ by 31-54% compared to control. This finding showed that proline accumulation was an important part of the physiological adaptation against Cd stress. Consequently, the accumulation of osmoprotective metabolites is considered a multifaceted

adaptive mechanism that not only maintains osmotic homeostasis in response to heavy metal stress but also stabilizes cellular structures and limits oxidative stress.

6.3. Organic Acids and Metal Chelation

Organic acids such as citric, malic, oxalic, fumaric and succinic acid form complexes with metal ions through their carboxyl groups and decrease free metal concentration, and hence limit the damage on protein, nucleic acid and membranes. The formed metal-organic acid complexes are sequestered in the cells, especially in vacuoles, and contribute to the metal detoxification (Hall, 2002; Sharma & Dietz, 2009). Organic acids are also secreted to rhizosphere from roots and affect the solubility, mobility, and uptake of metal ions by plants. The direction of this effect may change depending on the kind of metal and plant (Jones, 1998; Dakora & Phillips, 2002).

Changes in organic acid metabolism are a part of metabolic adaptation to heavy metal stress. Especially citric and malic acids work with other metal binding mechanisms such as phytochelatin and metallothionein and contribute to decrease free metal level in cytoplasm and the related oxidative stress (Hall, 2002; Sharma & Dietz, 2009).

While studies on this mechanism in Asteraceae family are limited, studies on *Helianthus annuus* showed that heavy metal applications have changed organic acid secretion from roots and levels of citric and malic acids (Saber et al., 1999). Moreover, external malic acid application was reported to decrease Cd toxicity in *H. annuus* and increase root activity, decrease H_2O_2 accumulation and increase adsorption of Cd in roots and limits transfer to shoots (Hawrylak-Nowak et al., 2015). These findings show that organic acids can contribute to heavy metal tolerance by metal chelation, subcellular sequestration and limitation of oxidative stress.

6.4. Phenolic Compounds and Flavonoids

Heavy metal stress causes over production of reactive oxygen species in plants and causes lipid peroxidation, protein oxidation and DNA damage. To limit these damages, plants increase the production of non-enzymatic antioxidants

such as phenolic compounds and flavonoids, besides the enzymatic antioxidants. Phenolic compounds can directly neutralize the free radicals by their hydroxyl groups, form complexes with transition metals and limit lipid peroxidation and help protect the cellular structures. Moreover, activation of phenylpropanoid pathway which is the basic regulator of phenolic metabolism is accepted as one of the most significant components of metabolic adaptation of plants against oxidative stress (Michalak, 2006).

Flavonoids, a significant subgroup of phenolic compounds, play a role in antioxidant system and also act as metal chelator. Hydroxyl groups in flavonoids can form complexes with Cd, Cu, Fe and Pb ions; these complexes limit the damage caused by free metal ions and also increase the capacity of ROS scavenging capacity and hence support the balance of cellular redox. Under heavy metal stress, increase in the levels of enzymes such as phenylalanine ammonium lyase, calcon synthase and other phenylpropanoid pathway enzymes which take place in biosynthesis of flavonoids was reported. As a result, accumulation of flavonoid and other phenolic metabolites fastens up. This metabolic reprogramming is evaluated as an important component of the antioxidant defense system (Dixon & Paiva, 1995; Goncharuk & Zagoskina, 2023; Michalak, 2006).

Studies supporting the roles of phenolic compounds in heavy metal tolerance in Asteraceae family are increasing day by day. For instance, in *Carlina acaulis* plants exposed to 0.1 μM Cd for 85 days, the ac cumulation of chlorogenic acid in shoots was shown to increase more than 9.4 times compared to control, while it was shown to increase 3.2 times in roots. Moreover, 3,5-di-*O*-cafeoilcinic acid concentration in shoots were shown to increase by 2.5 times. Researchers stated that the increase in 3,5-di-*O*-cafeoilcinic acid was related with the total phenolic compound content and increase in antioxidant capacity; therefore, long term Cd stress was stated to support enhancing antioxidant defense systems of the plant (Dresler et al., 2020).

Similarly, there was a strong correlation between Cd content in leaves and total phenolic compound in *Taraxacum linearisquameum.* There results revealed that phenolic metabolites are part of important defense mechanism against Cd accumulation in plants, and they play efficient role in biochemical response against heavy metal stress. In general, phenolics and flavonoids are among the basic secondary metabolites supporting antioxidant defense

capacity, decreasing the oxidative damage caused by heavy metals and formation of heavy metal tolerance in Asteraceae family (Kováčik et al., 2016).

6.5. Role of Metabolic Plasticity in Heavy Metal Tolerance

Metabolic plasticity is the reprogramming capacity of the metabolic networks of the plants to adapt to the changing environmental conditions. Under heavy metal stress this process includes coordinated reprogramming of carbon, nitrogen, energy production, redox balance, antioxidant defense, and secondary metabolism pathways. This reprogramming provides the protection of cellular homeostasis and the balance between growth and defense (Obata & Fernie, 2012; Sharma & Dietz, 2009).

Heavy metal toxicity suppresses photosynthesis and carbon assimilation and causes changes in energy metabolism, which brings the need to regulate alternative metabolic pathways such as glycolysis, TCA cycle and pentose phosphate pathway. While NADPH produced especially by pentose phosphate pathway provides the necessary reductive power for the functionality of antioxidant systems, byproducts of TCA cycle are used in the production of amino acids and other defense metabolites (Kruger & Von Schaewen, 2003; Obata & Fernie, 2012). Moreover, changes in proline, glutamate, GABA and other amino acids contribute to osmotic regulation, ROS detoxification, metal chelation and stress signalization. Especially the link between glutamate metabolism and glutathione biosynthesis shows the coordination between the metabolic pathways in metal tolerance (Hasanuzzaman et al., 2020).

Metabolic reprogramming encompasses the biosynthesis of phenolic compounds, flavonoids, terpenoids and other secondary metabolites. These compounds contribute to removal of ROS, making metal ions form complexes and protection of cellular structures and support heavy metal tolerance (Sharma & Dietz, 2009).

These metabolic responses have been shown in Asteraceae family, especially on *Helianthus annuus*. LC-QTOF-MS/MS based metabolomic analysis on *H. annuus* roots exposed to Cr(VI) stress detected significant changes in phenolic compounds, sesquiterpene lactones and various secondary metabolite groups. The results revealed that chromium stress reprogrammed secondary metabolism in *H. annuus* and formed an adaptive defense response (Gonzalez Ibarra et al., 2017).

As a result, heavy metal tolerance is based on not only the change of a single metabolite, but rather coordinated reprogramming of carbon, nitrogen, energy and secondary metabolism pathways. Metabolomic approaches such as GC-MS, LC-MS and NMR provide a holistic understanding of these complex metabolic changes and contribute to the evaluation of molecular mechanisms of heavy metal tolerance and the phytoremediation potential of plants (Obata & Fernie, 2012; Anjitha et al., 2023).

7. CONCLUSION

Heavy metal contamination represents a major environmental challenge because of the persistence of metals in soil and their ability to interfere with essential physiological and cellular processes in plants. The responses of plants to heavy metal stress are complex and highly dependent on the metal type, concentration, exposure duration, plant genotype, and environmental conditions. The evidence reviewed in this chapter demonstrates that members of the Asteraceae family possess a wide range of physiological, biochemical, and molecular mechanisms that enable them to tolerate, accumulate, or restrict the movement of toxic metals within their tissues.

Heavy metal tolerance in Asteraceae cannot be attributed to a single defense mechanism. Instead, it results from the coordinated operation of processes beginning in the rhizosphere and continuing through root uptake, intracellular transport, chelation, sequestration, and tissue-specific metal distribution. Regulation of metal bioavailability by soil properties, root exudates, and rhizosphere microorganisms can influence the amount of metal entering the plant. Following uptake, cell wall binding, selective transport, phytochelatin and metallothionein-mediated chelation, and vacuolar sequestration contribute to reducing the concentration of free metal ions in the cytoplasm. The activity and regulation of transporter families such as ZIP, NRAMP, HMA, MTP/CDF, and ABC therefore represent important components of metal homeostasis and tolerance.

At the physiological level, heavy metal exposure commonly results in inhibition of root and shoot growth, disturbances in water relations, impairment of photosynthesis, alteration of mineral nutrient uptake, and reduction in biomass production. However, tolerant Asteraceae species can partially maintain these processes through efficient metal retention and

compartmentalization, regulation of root-to-shoot translocation, and preservation of photosynthetic and nutritional functions. Thus, the maintenance of growth, water balance, photosynthetic activity, and nutrient homeostasis may serve as important indicators for evaluating differences in metal tolerance among species and genotypes.

Oxidative stress is another central component of the heavy metal response. Excess metal exposure can enhance ROS generation and consequently damage membrane lipids, proteins, nucleic acids, and photosynthetic structures.

Asteraceae species counteract this stress through coordinated enzymatic and nonenzymatic antioxidant systems, including superoxide dismutase, catalase, ascorbate peroxidase, glutathione-related enzymes, ascorbic acid, glutathione, carotenoids, phenolics, and flavonoids. The effectiveness of these responses appears to be closely associated with the ability of tolerant plants to maintain cellular redox homeostasis and limit oxidative damage.

Importantly, heavy metal tolerance also involves substantial metabolic reprogramming. Changes in carbon and nitrogen metabolism, energy production, amino acid metabolism, organic acid metabolism, and secondary metabolite biosynthesis allow plants to redirect resources from growth toward defense and detoxification. The accumulation of proline and other osmoprotective metabolites, enhanced glutathione–phytochelatin metabolism, organic acid-mediated metal chelation, and increased production of phenolic compounds and flavonoids collectively contribute to maintaining cellular homeostasis under stress. Therefore, metabolic plasticity should be considered a fundamental component of heavy metal tolerance rather than a secondary consequence of metal exposure.

The diversity of Asteraceae, together with the contrasting tolerance and accumulation strategies observed among its members, makes this family particularly valuable for biomonitoring and phytoremediation. Species such as *Helianthus annuus*, *Berkheya coddii*, *Taraxacum*, *Artemisia*, *Centaurea*, and *Tagetes* illustrate different combinations of high biomass production, metal accumulation, metal exclusion, and physiological tolerance. However, successful application of Asteraceae species in phytoremediation requires consideration not only of their total metal accumulation but also of biomass production, root-to-shoot translocation, metal bioavailability, tissue

distribution, and the stability of accumulated metals.

Overall, heavy metal tolerance in Asteraceae is best understood as an integrated response involving rhizosphere interactions, metal transport and compartmentalization, physiological adjustment, antioxidant defense, and metabolic plasticity. Future research combining physiological measurements with transcriptomics, metabolomics, and other molecular approaches will be valuable for identifying the regulatory networks underlying species- and genotype-specific tolerance. Such integrated knowledge may support the selection or improvement of Asteraceae species for biomonitoring and environmentally sustainable phytoremediation of metal-contaminated ecosystems.

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CHAPTER 9

ARTIFICIAL LIGHTING-ASSISTED SPEED BREEDING IN VEGETABLE BREEDING: DEVELOPMENTS, APPLICATIONS AND FUTURE PERSPECTIVES

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1. INTRODUCTION

Plant breeding today is no longer limited to conventional breeding methods based solely on hybridization and selection; rather, it has evolved into an integrated concept in which classical approaches are combined with mutation- and chromosome modification-based methods, tissue culture and haploid technologies, genetic engineering, genomic selection, genome editing, high-throughput phenotyping, and speed breeding approaches that accelerate the breeding process.

One of the major factors limiting the rate of genetic gain in plant breeding is the need to complete numerous generations to develop new cultivars. In conventional hybridization and selection methods, the dependence of each generation on the natural growing season can cause breeding programs to take many years, particularly in plant species with long life cycles. Therefore, throughout the development of plant breeding, it has become necessary not only to improve selection accuracy but also to develop methods that shorten the interval between generations.

In this context, speed breeding is a complementary breeding approach in which environmental variables such as photoperiod, light intensity, light spectrum, and temperature are managed under controlled conditions to shorten the generation interval and, when necessary, are integrated with early seed harvest and other generation-advancement techniques. In particular, advances in artificial lighting technologies have increased the applicability of this approach by reducing the dependence of plant development on season and natural day length.

In this review, the historical development of the speed breeding approach is briefly discussed, and the role of artificial lighting in rapid generation advancement, with particular emphasis on recent applications developed for vegetable species, is evaluated. The effects of photoperiod, light intensity, and spectral composition on generation time; the need for species- and genotype-specific protocols; integration with modern breeding technologies; limitations of the method; and future research directions are discussed together.

2. HISTORICAL DEVELOPMENT OF THE SPEED BREEDING APPROACH

2.1. Early approaches to shortening generation time

One of the earliest approaches toward this goal was shuttle breeding, which is based on exploiting suitable growing seasons in different geographical regions to obtain multiple generations within the same year. Borlaug's (1958) application of this approach in wheat breeding, by transferring breeding material between growing locations at different altitudes and latitudes, represented an important milestone by demonstrating that generation time could be shortened through management of environmental conditions. Shuttle breeding nevertheless has several disadvantages, including high costs and logistical requirements, difficulty in identifying suitable locations, genotype × environment interactions, quarantine/material-transfer problems, and dependence on environmental conditions.

Single Seed Descent (SSD) and Rapid Generation Advance (RGA), developed later, focused on advancing segregating populations to later generations as rapidly as possible. In SSD, generally one seed is taken from each plant to advance the population, with the aim of rapidly achieving homozygosity rather than conducting intensive phenotypic selection in early generations. RGA aims to shorten the plant life cycle and obtain more generations per year through controlled temperature, photoperiod, and growing conditions. Thus, generation advancement began to become less dependent on growing seasons. However, although SSD and RGA shorten generation-advancement time, they do not eliminate the overall selection and cultivar-development process. In advanced generations, traits such as yield, quality, disease resistance, and environmental adaptation still need to be evaluated under field conditions. Major disadvantages of SSD include line loss due to reliance on a single seed, limited early selection, and the need for several generations. Disadvantages of RGA include controlled-environment and energy costs, genotype-dependent responses, and the inability of controlled-environment results to fully represent field conditions.

Another important development in reducing breeding time has been the use of haploid and doubled haploid (DH) technologies. DH technology enables fully homozygous lines to be obtained in a single generation through chromosome doubling of the haploid genome, thereby substantially shortening

the pure-line development process that otherwise requires numerous generations of conventional selfing. While DH primarily reduces the time required to reach homozygosity, SSD, RGA, and later speed breeding approaches focus on shortening the physical duration of the generation cycle. However, the success of DH production can vary considerably among plant species, cultivars, and even genotypes within the same species. Some genotypes respond only weakly, whereas others fail to produce haploids altogether. Low regeneration rates may also be a problem. Because not all haploid cells obtained from anther, microspore, or ovule culture develop into healthy and fertile plants, large amounts of starting material may be required.

Accordingly, approaches such as shuttle breeding, SSD, and doubled haploidy are more appropriately described not as earlier forms of speed breeding itself, but as generation-acceleration or breeding-cycle-shortening approaches used before or in combination with speed breeding.

The origins of Rapid Generation Advance (RGA) can be traced to Goulden's work in 1939. Initially intended to rapidly advance segregating populations and accelerate homozygosity, the approach began to be applied more systematically in plant breeding following developments in the 1960s and the wider adoption of Single Seed Descent (SSD). The refinement of RGA through controlled environments, early seed harvest, and cultivation techniques that shorten generation time subsequently provided a foundation for the emergence of modern speed breeding (Goulden, 1939; Grafius, 1965; Collard et al., 2017; Cobb et al., 2019).

2.2. Transition from controlled-environment studies to modern speed breeding

Studies on growing plants under artificial light date back to the late nineteenth and early twentieth centuries. Early research demonstrated that plants could be grown under artificial illumination independently of natural sunlight. Subsequent studies showed that extending the photoperiod could alter flowering and developmental rates in some plants. This knowledge formed the physiological basis of contemporary speed breeding protocols.

NASA studies should not be regarded as the direct invention of modern speed breeding, but rather as an important precursor demonstrating that the combined use of controlled environments, long photoperiods, and rapidly

developing genotypes could substantially shorten the plant life cycle. To enable rapid and productive crop cultivation under the limited space and resources of long-duration space missions, NASA and Utah State University researchers investigated wheat growth and photosynthetic performance under controlled-environment conditions beginning in the 1980s. These studies examined how controlled regulation of light intensity, photoperiod, temperature, and other environmental factors affected plant growth and productivity (Bugbee and Salisbury, 1988).

One important outcome of these studies was the development of wheat genotypes with very short life cycles. The fully dwarf, hard red spring wheat cultivar 'USU-Apogee', developed by the Utah Agricultural Experiment Station in collaboration with NASA, was released in 1996. Under controlled long-day conditions, Apogee can flower in approximately 25–30 days without vernalization and can complete one generation in two months or less. Under suitable controlled conditions, this theoretically allows up to six generations per year (Li et al., 2017).

Later studies showed that Apogee's extremely short life cycle results not only from environmental conditions but also from genetic characteristics controlling flowering time. Li et al. (2017) demonstrated that Apogee flowers early under long day and controlled temperature conditions, attributing this to a combination of early flowering alleles associated with vernalization and photoperiod responses. Thus, NASA-supported research showed that combining a suitable genotype with optimized lighting and environmental conditions can substantially reduce generation time.

Although these studies were initially conducted not to accelerate plant breeding programs but to investigate crop production in controlled life-support systems and space environments, the accumulated knowledge provided an important basis for later speed breeding approaches. In particular, demonstrating that plant development could be accelerated under long or continuous photoperiods, high light levels, and controlled temperature closely corresponds to the environmental manipulations used in modern speed breeding systems. Today, speed breeding adapts these principles to breeding programs to obtain more generations per unit time and thereby accelerate genetic gain.

The next major step was the use of this approach not only for plant physiology or space agriculture, but directly for rapid advancement of breeding

material. In the early 2000s, when the term 'speed breeding' began to be used, researchers at the University of Queensland employed it to describe methods developed to accelerate wheat breeding. From the 2000s onward, combining controlled-environment conditions with generation-acceleration techniques became increasingly important. In addition to optimizing environmental factors such as photoperiod, temperature, and light intensity, early harvesting and rapid germination of immature seeds were shown to further reduce generation time. For example, Zheng et al. (2013) reported that combining controlled-environment conditions with embryo culture in spring wheat could achieve approximately eight generations per year. Studies have shown that by controlling and modifying environmental conditions, the generation time is not limited by the plant's natural growing season and can be significantly shortened.

Hickey et al. (2017) reported that accelerated breeding can shorten generation time under controlled conditions within a directly applied plant breeding program. The researchers combined a modified backcrossing approach with multiple disease phenotyping and speed breeding for rapid generation advancement. Accelerated growth conditions enabled some non-selected generations to be advanced more rapidly, while controlled phenotypic selection was performed in generations evaluated for disease resistance. The study demonstrated that speed breeding is not merely an experimental method for increasing the number of generations, but can be integrated with conventional breeding practices such as backcrossing, phenotypic selection, disease-resistance screening, and gene pyramiding. It thereby showed that speed breeding can facilitate the rapid transfer of multiple target traits into well-adapted elite cultivars and the combination of superior traits within the same genetic material. In this respect, Hickey et al. (2017) is regarded as one of the important studies marking the transition from rapid generation advancement under controlled conditions to modern, application-oriented speed breeding systems.

One of the most important milestones in defining speed breeding in its modern sense and promoting its international adoption was the study by Watson et al. (2018), published in Nature Plants under the title 'Speed breeding is a powerful tool to accelerate crop research and breeding'. Using controlled temperature and particularly extended photoperiods, the researchers demonstrated that up to six generations per year could be achieved in wheat,

barley, chickpea, and pea. The fact that the resulting plants showed normal development demonstrated that this method could be used not only for generational advancement but also for phenotypic selection.

As a result, accelerated breeding began to evolve from an experimental technique used by a limited number of research groups into a standardized breeding platform.

3. ROLE OF ARTIFICIAL LIGHTING IN SPEED BREEDING

The continuous development of artificial lighting technologies has laid the foundation for rapid reproduction by enabling the control of light conditions that regulate plant growth and development. Light is not only an energy source for photosynthesis but also a crucial signal regulating developmental processes such as flowering, photoperiodism, and photomorphogenesis. With artificial light sources, by controlling the duration, intensity, and spectral composition of light, photosynthetic activity and plant development are directed, and the transition to flowering accelerated.

3.1. Photoperiod

Particularly in long-day plants, extending the photoperiod through artificial lighting promotes earlier flowering and consequently shortens the seed-to-seed generation interval. However, because the photoperiodic requirements of short-day and day-neutral plants differ, modern speed breeding systems require not simply longer illumination but the establishment of an appropriate species-specific light regime. Artificial lighting therefore enables plant developmental cycles to be controlled independently of natural day length and seasonal variation, allowing more generations to be obtained within the same year (Watson et al., 2018; Wanga et al., 2021).

3.2. Light intensity and controlled growing environments

Artificial lighting has also contributed to making speed breeding largely independent of season and geographical location. Establishing reproducible year-round growing conditions in growth chambers, controlled-environment facilities, and artificially illuminated greenhouses makes it possible to obtain multiple generations within a single year. Various light sources, including fluorescent, metal-halide, high-pressure sodium, and more recently LED

systems, can be used for rapid generation advancement. The development of energy-efficient and spectrally tunable LED systems has further increased the applicability of speed breeding by enabling more precise control of lighting conditions.

3.3. Light spectrum and the R:FR ratio

One important advantage of LED systems is that the light spectrum can be adjusted according to plant requirements. In speed breeding protocols, light duration, intensity, and spectral composition can be optimized according to the plant species and developmental stage. Plant growth is also affected by the spectral composition and photoperiod of light. LED technology allows for the application of red, blue, and other wavelengths in different ratios, enabling more controlled regulation of photosynthesis, photomorphogenesis, and flowering.

3.4. Advantages provided by LED technology

LEDs offer significant advantages over traditional light sources due to their high energy efficiency, long lifespan, and lower heat load, making them ideal for rapid growth systems utilizing long photoperiods. Watson et al. (2018) reported that the potential cost of LED-based supplemental lighting is low, while Ghosh et al. (2018) studied LED analyses demonstrating rapid growth protocols in growing media. Overall, LED technology excels in energy use while also enabling controlled photoperiods and precise spectrum deployment.

4. ARTIFICIAL LIGHTING-ASSISTED SPEED BREEDING APPLICATIONS IN VEGETABLES

Studies in vegetables indicate a progression from investigations of the effects of supplementary lighting on plant development toward species-specific speed breeding protocols in which photoperiod and spectrum are deliberately adjusted to reduce generation time.

4.1. Tomato

One of the important early studies on modifying developmental duration through artificial lighting in vegetable crops was conducted by Demers et al. (1998) in greenhouse tomato. The researchers investigated the effects of the duration of supplementary lighting on plant development and fruit yield in

Lycopersicon esculentum. Although this study did not directly use the concept of 'speed breeding', it can be considered part of the physiological foundation for later controlled-environment studies because it experimentally demonstrated that photoperiod and supplementary light could modify tomato development and reproductive behavior.

The importance of this study lies in demonstrating that, in vegetables, not only light quantity but also the timing and duration of light application during the day are controllable cultivation factors affecting development. However, the primary objective at that time was not to shorten generation time and accelerate the breeding cycle, but to optimize plant development and yield in greenhouse production. Therefore, the study is more appropriately categorized as part of the controlled-light physiology foundation of modern speed breeding rather than as speed breeding itself.

A different approach in the development of tomato speed breeding research was presented by Gimeno-Páez et al. (2025). The researchers evaluated pot volume, cold treatment, water stress, mineral nutrition, and particularly embryo rescue to reduce generation time in two tomato cultivars with determinate and indeterminate growth habits. The use of embryo rescue reduced the need to wait for seeds to reach full physiological maturity on the fruit and shortened generation time by approximately 8.7–11.6 days, depending on the cultivar. Combining agronomic practices with embryo rescue demonstrated the potential to increase tomato generation turnover from approximately three to four generations per year.

The significance of this study for the artificial-lighting literature is that it demonstrated that generation time in vegetables is not determined solely by the period to flowering. Fruit development, physiological seed maturation, and attainment of germinability also represent important time constraints. Therefore, an effective vegetable speed breeding system should simultaneously target early flowering, rapid fruit development, and early seed acquisition.

One of the most direct recent developments in artificial lighting-based speed breeding in vegetables was reported by Hu et al. (2025) in tomato. The study aimed to determine and optimize an appropriate speed breeding light recipe in terms of light intensity and photoperiod for two tomato cultivars under plant factory conditions.

In light-intensity experiments, values of approximately 300–400 μmol m^{-2} s^{-1} were found to be suitable for development and seed maturation. Approximately 350 μmol m^{-2} s^{-1} was subsequently used as the baseline light intensity in photoperiod experiments.

Researchers also determined in this study how quickly germinating seeds could be obtained. It was reported that germinating seeds were obtained in approximately 60-63 days in tomato varieties. This theoretically corresponds to about six generations per year. Studies on tomatoes have focused not only on the effect of generation time on flowering, but also on the cycle time of fruit development, physiological seed maturation, etc.

4.2. Pepper

One of the important studies directly optimizing the artificial light environment in vegetables was carried out by Liu et al. (2022) in peppers. They reported that extending the photoperiod to 20 hours advanced flowering by approximately 2-3 days, and that extending the photoperiod alone did not accelerate fruit and seed development to the same extent. This shows that providing light for a long time is insufficient for rapid vegetable reproduction. The study also reported that the addition of far-red light increased the rate of fruit ripening and the germination rate of seeds was high.

Another important study in accelerated vegetable cultivation research was conducted by Choi et al. (2023). The researchers not only developed an agronomic acceleration protocol for *Capsicum annuum* but also investigated at the genetic and molecular levels why light accelerates flowering.

Light/dark regimes of 12/12, 16/8, 20/4, and 24/0 h were compared. Increasing the photoperiod to 20 h markedly accelerated flowering. Different R ratios were then tested, and increasing the amount of far-red light was found to accelerate the transition from the vegetative to the reproductive phase.

The most effective controlled-environment treatment consisted of 20 h light/4 h dark combined with a low R:FR ratio (0.3). Under these conditions, the first mature fruit was obtained after approximately 170 days in the control group, whereas this period was reduced to approximately 95 days under the Epp+FR treatment, advancing the first harvest by about 75 days.

The researchers subsequently transferred the system to the greenhouse, where LED supplementation of natural light was used to establish a 20-h

photoperiod and an appropriate R ratio. Under greenhouse conditions, flowering took approximately 51 days under normal treatment but was reduced to approximately 21 days under extended photoperiod + FR treatment. The seed-to-seed cycle was shortened to approximately 110 days.

Choi et al. (2023) did not evaluate the effect of light solely at the phenotypic level; they also performed GWAS and gene-expression analyses and identified AP2, WOX4, FT, and GI as candidate genes associated with flowering. Photoperiod and FR treatments were shown to alter the expression of genes particularly associated with flowering. The researchers described this as the first study investigating the light-dependent flowering response at the gene-expression level in a pepper speed breeding system. Thus, artificial lighting in speed breeding began to be considered not merely as an environmental energy input, but as a developmental signal regulating flowering genes.

When the studies by Liu et al. (2022) and Choi et al. (2023) are evaluated, it is concluded that simply extending the photoperiod is not sufficient for seed-to-seed transition, and that light intensity, and especially the R:FR ratio, should be optimized along with the photoperiod to shorten the seed-to-seed transition time.

4.3. Other vegetable species and plant factory approaches

Sinyavina et al. (2023) conducted a study to select genotypes suitable for artificial light production media based on the growth time, yield and morphological characteristics of small radish and daikon materials under artificial light in *Raphanus sativus*. This study is also important in terms of integrating with plant factory/artificial light culture systems. It indicates that future speed breeding programs in leafy and root vegetables may select not only for rapid generation turnover but also for adaptation to controlled environments.

He et al. (2024) comprehensively evaluated the use of plant factory systems for speed breeding in vegetables. The researchers proposed simultaneous control not only of photoperiod but also of light intensity, light spectrum, temperature, CO_2 concentration, mineral nutrition, and planting density.

This work represents an important conceptual shift in vegetable speed breeding. Speed breeding is increasingly regarded not merely as a method of extending photoperiod in a growth chamber, but as a fully controlled cultivation and breeding platform. Because plant factories permit cultivation largely independent of the external environment, continuous year-round generation advancement may theoretically become possible.

The researchers also regarded integration of these systems with genome editing, genomic selection, high-throughput genotyping, and phenotyping as an important future direction in vegetable breeding.

4.4. Comparative summary of studies in vegetables

The chronological development and major contributions of artificial lighting-assisted speed breeding studies in vegetables are summarized in Table 1.

Table 1. Artificial lighting-assisted speed breeding studies in vegetables

Reference	Species	Light/environmental treatment	Other approach	Main result	Major contribution
Demers et al. (1998)	Tomato	Supplementary light; duration of application	–	Plant development and fruit yield responded to lighting duration	Early foundation for controlled-light physiology in vegetables
Liu et al. (2022)	Pepper	20-h photoperiod; light intensity; R:FR	–	Earlier flowering; potential for approximately 4 generations/year	Combined optimization of photoperiod + intensity + spectrum
Choi et al. (2023)	Pepper	20/4 h; low R:FR (0.3)	GWAS and gene expression	First mature fruit reduced from ~170 to 95 days; seed-to-seed ~110 days	Linking light response with its molecular basis
Sinyavina et al. (2023)	Radish/daikon	Fully artificial-light conditions	Genotype selection	Evaluation of genotypes	Breeding approach for

				suitable for controlled environments	adaptation to plant factory conditions
He et al. (2024)	Vegetables	Light intensity + spectrum + photoperiod; temperature and CO_2 control	Nutrition and planting density	Framework for year-round controlled generation advancement	Speed breeding considered as a fully controlled platform
Gimeno-Páez et al. (2025)	Tomato	Agronomic practices	Embryo rescue	Generation time shortened by 8.7–11.6 days; potential increase from 3 to 4 generations/year	Targeting the seed-maturation bottleneck
Hu et al. (2025)	Tomato	~350 µmol m^{-2} s^{-1}; 20-h photoperiod	Plant factory	Germinable seed in ~60–63 days; theoretically ~6 generations/year	Direct optimization of tomato light recipe

5. SPECIES- AND GENOTYPE-SPECIFIC OPTIMIZATION OF SPEED BREEDING PROTOCOLS

Nevertheless, results obtained in vegetables demonstrate that there is no single universal speed breeding light recipe. The 22-h photoperiod used in cereals cannot be directly applied to all vegetable crops. In species such as tomato and pepper, important interactions occur among photoperiod, R:FR ratio, light intensity, and genotype; excessively long photoperiods may even cause physiological injury in some tomato genotypes. Modern vegetable speed breeding is therefore increasingly moving toward the development of species- and even genotype-specific light recipes.

Rossi et al. (2026) investigated whether the long photoperiods used in speed breeding could be optimized according to barley genotype. The study showed that ppd-H1 genotypes under an approximately 20-h photoperiod and Ppd-H1 genotypes under a 16-h photoperiod could flower at rates similar to

those under the standard 22-h treatment. The ELF3 and PHYC alleles were also identified as key regulators of photoperiod sensitivity and early maturation. The study demonstrates that genotype-specific photoperiod adjustment can improve the efficiency of rapid breeding systems, offering a novel approach to developing climate change-resistant barley varieties.

The researchers emphasized that although speed breeding protocols have largely been implemented using a uniform 22-h light period, photoperiod requirements can vary substantially among genotypes. Determining photoperiod thresholds can avoid unnecessarily long lighting periods, thereby reducing electricity consumption and production costs. The combined evaluation of ELF3, PPD-H1, and PHYC was also reported to provide an important genetic basis for developing early barley cultivars capable of adapting to climate change.

By showing that 16- or 20-h photoperiods can be used according to genetic background instead of the conventional 22-h protocol, the study proposed new energy-saving speed breeding strategies. Explaining the molecular basis of photoperiod response through PPD-H1, ELF3, and PHYC also provides important contributions both to speed breeding programs and to the development of climate-resilient barley cultivars.

When findings in vegetables are considered together with studies demonstrating genotype-specific photoperiod responses, it becomes clear that speed breeding protocols should not be based on a uniform lighting program. Optimum photoperiod, light intensity, and spectral composition should be determined according to the species' photoperiodic characteristics, genotype, developmental stage, and the targeted breeding phase.

6. INTEGRATION WITH MODERN BREEDING TECHNOLOGIES

A major trend in the development of speed breeding during the 2020s has been its integration with other modern breeding technologies. Combining marker-assisted selection, genomic selection, high-throughput phenotyping, and genome editing with speed breeding aims not only to shorten generation time but also to increase the accuracy and efficiency of selection in each generation. In particular, the use of genomic selection in conjunction with accelerated breeding is considered important for increasing genetic gain per

time. Similarly, integrating CRISPR/Cas-based genome editing with accelerated breeding can be a useful method for rapidly advancing edited genotypes. Wanga et al. (2021) reported that accelerated breeding, when used in conjunction with other modern breeding techniques, would be a tool to enhance genetic progress.

Ćeran et al. (2024) comprehensively evaluated the use of genomic-assisted breeding methods in conjunction with accelerated breeding. The review reported that speed breeding under extended 20–22-h photoperiods and controlled-environment conditions shortened generation time by 2.5–5-fold and enabled five or more generations per year in many plant species. It further emphasized that combining marker-assisted selection, genomic selection, QTL mapping, and CRISPR/Cas-based gene editing with speed breeding improves selection accuracy, increases annual genetic gain, and substantially reduces the time required for cultivar development. Photoperiod manipulation and LED lighting were identified as fundamental components of genomics-assisted rapid plant breeding systems and were considered likely to play an important role in developing climate-resilient cultivars.

The researchers emphasized that one of the greatest bottlenecks in modern plant breeding is not merely generating genetic variation, but evaluating that variation rapidly. While speed breeding shortens generation time through optimization of environmental conditions, genomic technologies allow suitable individuals to be selected early at the molecular level. When the two approaches are combined, both selection accuracy increases and cultivar-development time is substantially reduced. Photoperiod manipulation and LED lighting were particularly identified as key environmental components of genomics-assisted rapid breeding systems. The study indicates that not only rapid generation advancement but also the combined use of genomic selection, marker-assisted selection, CRISPR/Cas-based genome editing, and high-throughput phenotyping can substantially increase annual genetic gain. Future integration of AI-assisted phenotyping, automation, and omics technologies into speed breeding systems is expected to further accelerate the development of climate-resilient, high-yielding cultivars.

Singh et al. (2026) comprehensively examined how conventional and molecular breeding methods can be integrated for sustainable crop development in vegetable breeding and evaluated the role of speed breeding

within this integration. The study noted that conventional phenotype-based selection is time-consuming because of long generation intervals, whereas molecular breeding methods enable more precise selection of target genes. The review emphasized that optimizing photoperiod, light intensity, light spectrum, temperature, and CO_2 levels under controlled-environment conditions can substantially shorten generation time in vegetable crops. In particular, it reported that 4–6 generations per year may be achieved in vegetables such as tomato, pepper, lettuce, and cabbage, thereby accelerating gene pyramiding, gene introgression, and molecular-marker validation. Combining immature embryo culture, early seed germination, genomic selection, marker-assisted selection, and CRISPR/Cas-based genome editing with speed breeding was also reported to substantially reduce the time required to develop climate-resilient, high-yielding, high-quality cultivars. The authors concluded that speed breeding should be regarded not as a stand-alone breeding method but as a strategic generation-advancement tool that complements conventional and molecular approaches and improves the efficiency and accuracy of vegetable breeding programs.

In this integrated approach, speed breeding generates new generations more rapidly, while genomic and molecular tools allow suitable individuals to be selected earlier and more accurately. The primary objective is therefore not merely to obtain more generations per year, but to increase genetic gain per unit time.

7. LIMITATIONS AND PRACTICAL CHALLENGES

Although speed breeding can considerably shorten the breeding process, its effectiveness may vary depending on the plant species, genotype, and available growing infrastructure. In particular, establishing controlled environments and operating artificial lighting systems for extended periods can increase both initial investment and operating costs. Although LED systems can reduce energy consumption compared with conventional lighting sources, lighting costs remain an important consideration in breeding programs involving large populations.

Another important issue is that results obtained under controlled environments may not fully reflect plant performance under field conditions. In the field, plants are exposed to variable conditions resulting from the combined

effects of temperature, light, water availability, and other environmental factors. Therefore, breeding materials rapidly advanced through speed breeding should subsequently be evaluated in field trials for traits such as yield, quality, disease resistance, and environmental adaptation. In addition, the limited space available in growth chambers and other controlled-environment facilities may restrict the number of plants that can be evaluated simultaneously. This should be taken into account particularly when working with large and genetically diverse populations.

In vegetable crops, shortening the generation cycle cannot be achieved solely by inducing earlier flowering. After flowering, additional time is required for fruit development, physiological seed maturation, and the acquisition of sufficient seed germination capacity. These stages should therefore also be considered when attempting to reduce the overall generation time. Furthermore, the very long photoperiods commonly used in cereal speed breeding cannot necessarily be transferred directly to vegetable crops. Some tomato genotypes, for example, may exhibit adverse physiological responses to prolonged photoperiods. Consequently, speed breeding protocols developed for vegetable crops should be evaluated not only in terms of the reduction in generation time, but also with respect to the effects of the light regime on plant development, energy requirements, and the economic sustainability of the system.

8. FUTURE PERSPECTIVES

Čupić et al. (2026) reported that the success of speed breeding does not depend solely on providing plants with longer light periods. Coordinated management of photoperiod, light spectrum, temperature, the species' photoperiodic response, and early seed harvest shortens generation-advancement time. In particular, an approximately 22-h light/2-h dark photoperiod combined with appropriate proportions of red and blue LED light plays an important role in accelerating vegetative and reproductive development. Under these conditions, up to six generations per year can be achieved in some crops such as wheat, barley, and chickpea.

Blinkov et al. (2025) comprehensively reviewed the environmental and biotechnological approaches used in speed breeding. The review reported that extended photoperiods of particularly 20–22 h, when combined with an

appropriate LED spectrum, temperature, and CO_2 management, shorten flowering time and enable 4–6 generations per year in many plant species. It also emphasized that integrating embryo culture, the use of immature seeds, and genomic selection with speed breeding substantially accelerates pure-line development, gene introgression, and molecular breeding. The study identified photoperiod manipulation as a core component of modern rapid plant breeding and highlighted the importance of developing species- and genotype-specific protocols for future breeding programs. The authors emphasized that speed breeding is not merely a method based on photoperiod extension, but an integrated cultivation system in which light quality, light intensity, temperature, CO_2, nutrition, and advanced biotechnological applications are optimized together. Advances in LED technology now make it possible to create species- and genotype-specific light recipes, thereby accelerating both plant development and breeding. Rather than relying on a single standard protocol, speed breeding protocols should therefore be tailored to plant species and genotype. Extended photoperiod, particularly when combined with LED lighting and temperature optimization, has emerged as a fundamental component of rapid breeding systems. Current applications integrating speed breeding with genomic selection, marker-assisted selection, CRISPR-based genome editing, and pure-line development provide a comprehensive framework for modern plant breeding.

In the future, vegetable speed breeding systems are expected to become more strongly integrated with full-spectrum and tunable LED lighting, automated environmental control, high-throughput phenotyping, genomic selection, marker-assisted selection, CRISPR/Cas-based genome editing, and artificial-intelligence-assisted decision systems. The success of this approach will be evaluated not only by shortened generation time, but also by the development of energy-efficient, species/genotype-specific protocols and validation of controlled-environment results through multi-location field trials.

9. CONCLUSION

Artificial lighting has evolved from being merely a production input supplementing insufficient natural light in speed breeding systems into an active breeding tool that regulates plant development and generation time through photoperiod, light intensity, and spectral composition. In particular, the

spectral control, low heat load, and energy efficiency provided by LED technology have increased the feasibility of rapid generation advancement in controlled environments.

Studies in vegetables clearly demonstrate that no single universal speed breeding protocol exists. Species and genotypes, particularly tomato and pepper, can respond differently to photoperiod, light intensity, and the R:FR ratio, while fruit and seed development may constitute major bottlenecks in total generation time. Therefore, future protocols should generally target the entire seed-to-seed cycle. Consequently, artificial light-assisted accelerated breeding in vegetables should be considered not as a standalone method, but as a complement to conventional and molecular breeding, accelerating these approaches. The development of specific light recipes, optimization of energy use, and integration with genomic selection, genome editing, high-throughput phenotyping, and automation will be key determinants of the effectiveness of this approach in future vegetable breeding programs.

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CHAPTER 10

THE PHYTOTOXIC EFFECT OF *Pistacia terebinthus* L. PLANT EXTRACT ON WEED AND CROP SPECIES

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1. INTRODUCTION

Weeds are among the major biotic stress factors limiting yield and quality in agricultural ecosystems. By competing with cultivated plants for water, light, nutrients, and space, weeds cause direct yield losses and may also inflict indirect damage through allelopathic interactions (Zohaib et al., 2016; Savić et al., 2025). Allelopathy is defined as the production and release of biochemical compounds by plants that affect the growth and development of surrounding organisms, thereby representing the chemical dimension of interplant competition (Rice, 1984; Inderjit & Duke, 2003). The allelochemicals involved in this process consist of various secondary metabolites, including phenolic acids, flavonoids, alkaloids, terpenoids, and quinones (Weston & Duke, 2003).

In modern agriculture, weed control relies largely on synthetic herbicides. However, the intensive and indiscriminate use of these chemicals has led to serious problems such as environmental pollution, degradation of soil and water resources, harm to non-target organisms, and the development of herbicide resistance (Baćmaga et al., 2024; Parven et al., 2025; Heap, 2023; Joseph et al., 2024). This situation has made it necessary to develop environmentally friendly and biologically based alternatives within the framework of sustainable agricultural practices. In this context, natural compounds derived from allelopathic plants are considered a promising source for the development of bioherbicides (Dayan et al., 2009; Macías et al., 2007). The allelopathic effects of plant extracts are directly related to the diversity and concentration of the secondary metabolites they contain. Phenolic compounds, in particular, can suppress plant growth through mechanisms such as inhibiting cell division, disrupting enzyme activities, and altering cell membrane integrity (Mushtaq & Fauconnier, 2024; Einhellig, 1995). In addition, the effects of allelochemicals may vary depending on the species of the target plant, its developmental stage, and environmental conditions (Inderjit, 2005).

Pistacia terebinthus L., a member of the Anacardiaceae family, is native to the Mediterranean region and Asia, and its intermediate forms are quite widespread in Türkiye. In Türkiye, particularly in the Taurus Mountains, it grows on dry rocky slopes, hills, and pine forests at elevations ranging from sea level to 1600 m. This species, which occurs naturally in the flora of Türkiye, is commonly known as “menengiç” and is used in traditional medicine for

antiseptic, anti-inflammatory, and digestive regulatory purposes (Özcan, 2004; Tunç & Sağlam Yılmaz, 2023). The rich phytochemical composition found in the fruits, leaves, and resins of *P. terebinthus* forms the basis of its biological activities. Various studies have emphasized that it is particularly rich in phenolic compounds, flavonoids, essential oils, and triterpenes, and that these compounds exhibit antioxidant, antimicrobial, and antifungal activities (Topçu et al., 2007; Tunç & Sağlam Yılmaz, 2023).

Although studies on the allelopathic potential of species belonging to the genus *Pistacia* are limited, some research has indicated that the secondary metabolites contained in these species may exert inhibitory effects on plant growth. In particular, the phenolic richness of leaf and fruit extracts suggests that these organs may possess significant allelopathic potential. Nevertheless, comparative studies examining the allelopathic effects of methanol extracts obtained from different organs of *P. terebinthus* remain very limited. Methanol is a commonly used solvent in plant extraction, as it enables the efficient extraction of a broad spectrum of polar and semi-polar compounds. For this reason, methanol extracts are frequently preferred in determining the allelopathic potential of plants (Sasidharan et al., 2011). The allelopathic effects of plant extracts are generally evaluated using parameters such as germination rate, root length, and shoot length, which are particularly important because they reflect the early stages of plant development.

In this context, the present study aimed to comprehensively evaluate the allelopathic effects of methanol extracts obtained from the fruits and leaves of *Pistacia terebinthus* on selected test plants. It is expected that the findings obtained from determining the inhibitory effects of these extracts on germination, root growth, and shoot growth will contribute to the development of natural and environmentally friendly bioherbicides and provide alternative solutions for sustainable agricultural practices.

2. MATERIALS AND METHODS

2.1. Plant Material

The principal plant material used in this study, *Pistacia terebinthus* (menengiç), was collected from Kahramanmaraş Province. The leaves and fruits of the collected plants were shade-dried. The dried plant materials were then ground into powder using an electric grinder. The

powdered materials were stored in paper bags under shaded conditions until used in the experiments.

2.2. Preparation of Methanol Extracts

A 100 g sample from each plant material was weighed and placed into 1 L Erlenmeyer flasks, and sufficient methanol was added to completely cover the material. The mixtures were shaken at 120 rpm on an orbital shaker at room temperature for 48 h. The extracts were subsequently filtered through filter paper. Methanol was removed by allowing it to evaporate at 32°C until a solid residue was obtained. The resulting extracts were stored at +4°C. For experimental use, the extracts were dissolved in DMSO + distilled water to prepare different concentrations (Control, 0.5, 1, 2, and 4 mg/mL) (Kadioglu and Yanar, 2004).

2.3. Effects of Plant Methanol Extracts on Seed Germination and Seedling Growth of Test Plants

The *in vitro* experiments were conducted in Petri dishes with a diameter of 9 cm. Two layers of drying paper were placed in each Petri dish, and 25 seeds of each test plant were evenly distributed. The prepared extracts from different plant parts (leaf and fruit) were applied at different concentrations (0.5, 1, 2, and 4 mg/mL), and 5 mL of each solution was added to the Petri dishes for moistening; distilled water was used as the control treatment. The Petri dishes were incubated at an average temperature of 24°C for 1–3 weeks. At the end of the incubation period, germination percentages, root lengths, and shoot lengths of the test plants were measured (Yılar et al., 2014). The experiment was carried out with four replicates and repeated twice.

2.4. Data Analysis

The significance levels of differences among treatments were determined by analysis of variance (ANOVA), and the means were compared using Duncan's multiple range test. Statistical analyses were performed using the SPSS 15 software package.

3. RESULTS

It was determined that the leaf and fruit methanol extracts of *Pistacia terebinthus* (menengiç) exerted allelopathic effects on seed germination, root growth, and shoot growth in different plant species. Examination of the effects

of leaf and fruit extracts at different doses on the germination and seedling development of test plants showed that allelopathic activity varied depending on the plant species, the plant part used, and the applied dose. In general, fruit extracts exhibited higher phytotoxic effects than leaf extracts. The results obtained for the test plants are presented in the tables below.

In the cultivated plant garden cress (*Lepidium sativum* L.), the leaf extract had a limited effect on seed germination; however, at the highest dose (4 mg mL^{-1}), it caused inhibition of 11.8% in germination rate, 62.5% in root length, and 14.1% in shoot length. The fruit extract exhibited a stronger effect; at the same dose, germination was suppressed by 55.9%, root growth by 82.3%, and shoot growth by 31.04%. These results indicate that root growth in garden cress was particularly sensitive to menengiç extracts (Table 1, Figure 1).

Table 1. Effect of the methanol extract of *Pistacia terebinthus* on the *Lebidium sativum* L. plant

Plant part	Doses (mg/ml)	Germination (%)	Root length (mm)	Shoot length (mm)
Leaf	**Control**	90.66^{a*}±5.3	82.71^{a}±3.9	22.13^{a}±0.3
	0.5	90.66^{a}±3.5	59.47^{ab}±15.4	25.74^{a}±3.4
	1	82.66^{a}±2.6	58.85^{ab}±2.2	25.77^{a}±2.8
	2	82.66^{a}±3.5	46.03^{bc}±2.7	21.12^{a}±1.9
	4	80.00^{a}±0.0	31.03^{c}±6.0	19.00^{a}±1.5
Fruit	**Control**	90.66^{a}±5.3	82.71^{a}±3.9	22.13^{a}±0.3
	0.5	77.33^{b}±3.5	36.30^{b}±2.3	18.03^{b}±0.3
	1	76.00^{b}±2.3	28.32^{c}±2.0	17.31^{bc}±0.7
	2	76.00^{b}±2.3	21.15^{cd}±0.3	16.05^{bc}±0.8
	4	40.00^{c}±4.0	14.65^{d}±1.8	15.26^{c}±1.0

* Means with different letters in the same column are significantly different according to Duncan's test at the $p < 0.05$ significance level

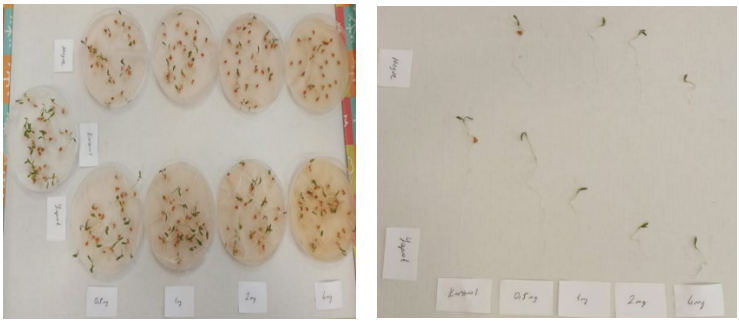

Figure 1. The Effect of a Methanol Extract of *P. terebinthus* on the Growth of *Lebidium sativum L.*

Wheat (*Triticum aestivum* L.) was found to be more resistant to the plant extract than garden cress, and its germination was relatively less affected by the extracts. At the 4 mg mL^{-1} dose of the leaf extract, only an 8.45% reduction in germination was observed, whereas the inhibition of root and shoot growth was determined to be 35.85% and 42.03%, respectively. The fruit extract, however, significantly suppressed root (66.69%) and shoot (63.73%) growth, while its effect on germination remained limited to only 5.63% (Table 2, Figure 2).

Table 2. Effect of the methanol extract of *Pistacia terebinthus* on the *Triticum aestivum* L. plant

Plant part	Doses (mg/ml)	Germination (%)	Root length (mm)	Shoot length (mm)
Leaf	**Control**	$94.66^{ab}\pm1.3$	$56.03^{a}\pm5.2$	$41.39^{a}\pm2.3$
	0.5	$96.00^{a}\pm0.0$	$41.58^{b}\pm2.3$	$31.90^{b}\pm1.9$
	1	$94.66^{ab}\pm5.3$	$39.44^{b}\pm2.2$	$31.41^{b}\pm1.6$
	2	$93.33^{ab}\pm1.3$	$38.66^{b}\pm1.2$	$30.53^{b}\pm1.6$
	4	$86.66^{b}\pm1.3$	$35.94^{b}\pm3.5$	$23.99^{c}\pm1.1$
Fruit	**Control**	$94.66^{a}\pm1.3$	$56.03^{a}\pm5.2$	$41.39^{a}\pm2.3$
	0.5	$92.00^{a}\pm1.3$	$29.02^{b}\pm3.4$	$20.49^{b}\pm1.1$
	1	$92.00^{a}\pm2.3$	$25.76^{bc}\pm1.7$	$17.13^{bc}\pm0.1$
	2	$90.66^{a}\pm2.3$	$20.14^{bc}\pm0.6$	$15.90^{c}\pm1.1$
	4	$89.33^{a}\pm1.3$	$18.66^{c}\pm1.3$	$15.01^{c}\pm0.2$

* Means with different letters in the same column are significantly different according to Duncan's test at the $p < 0.05$ significance level

Figure 2. Effect of the *P. terebinthus* methanol extract on the growth of *Triticum aestivum*

When considered overall, the menengiç plant extract exhibited a greater effect on weed species. In hollyhock (*Alcea rosea*), the most pronounced effect was observed on shoot growth. At the 4 mg mL^{-1} dose of the leaf extract, shoot

growth was completely inhibited (100%), while the inhibition rates for germination and root growth were determined to be 29.6% and 17.8%, respectively. The fruit extract, on the other hand, completely suppressed shoot growth (100% inhibition) at all doses of 1 mg mL^{-1} and above. In contrast, the maximum inhibition of germination and root growth remained limited to 18.8% and 28.4%, respectively (Table 3, Figure 3).

Table 3. Effect of the methanol extract of *Pistacia terebinthus* on the *Alcea rosea* plant

Plant part	Doses (mg/ml)	Germination (%)	Root length (mm)	Shoot length (mm)
Leaf	**Control**	$61.56^{a}\pm3.3$	$30.79^{ab}\pm3.5$	$19.78^{a}\pm0.5$
	0.5	$66.66^{a}\pm3.3$	$38.14^{ab}\pm3.3$	$17.41^{a}\pm1.4$
	1	$61.66^{a}\pm4.4$	$43.11^{a}\pm7.7$	$10.31^{ab}\pm6.0$
	2	$60.00^{a}\pm2.8$	$31.47^{ab}\pm3.5$	$8.69^{ab}\pm4.5$
	4	$43.33^{b}\pm4.4$	$25.30^{b}\pm2.0$	$0.00^{b}\pm0.0$
Fruit	**Control**	$61.56^{ab}\pm3.3$	$30.79^{a}\pm3.5$	$19.78^{a}\pm0.5$
	0.5	$68.33^{a}\pm5.0$	$27.19^{ab}\pm2.0$	$2.59^{b}\pm1.3$
	1	$50.0^{b}\pm4.4$	$25.28^{ab}\pm1.5$	$0.0^{c}\pm0.0$
	2	$50.0^{b}\pm5.0$	$23.77^{ab}\pm2.5$	$0.0^{c}\pm0.0$
	4	$50.0^{b}\pm2.8$	$22.06^{b}\pm1.4$	$0.0^{c}\pm0.0$

*Means with different letters in the same column are significantly different according to Duncan's test at the $p < 0.05$ significance level

Figure 3. Effect of the methanol extract of *P. terebinthus* on the growth of *Alcea rosea*

Purslane (*Portulaca oleracea*), another weed species, has been identified as one of the species most sensitive to menengiç extracts. At the highest dose of the leaf extract, germination, root, and shoot development were inhibited by 25.5%, 75.02%, and 34.05%, respectively. In applications of the fruit extract, these rates were calculated as 29.77%, 73.52%, and 53.25%, respectively. The high level of inhibition observed in root development, in particular, indicates

that the extracts have a significant phytotoxic effect on seedling growth (Table 4).

Table 4. Effect of the methanol extract of *Pistacia terebinthus* on *Portulaca oleracea* L.

Plant part	Doses (mg/ml)	Germination (%)	Root length (mm)	Shoot length (mm)
Leaf	**Kontrol**	$62.66^{a}\pm4.8$	$31.27^{a}\pm2.2$	$20.41^{a}\pm0.5$
	0.5	$62.66^{a}\pm5.3$	$24.98^{b}\pm1.6$	$18.15^{ab}\pm2.1$
	1	$62.66^{a}\pm1.3$	$22.35^{bc}\pm2.7$	$16.52^{ab}\pm1.8$
	2	$57.33^{a}\pm3.5$	$17.50^{c}\pm0.6$	$15.35^{b}\pm0.8$
	4	$46.66^{a}\pm7.4$	$7.81^{d}\pm0.0$	$13.46^{b}\pm0.8$
Fruit	**Kontrol**	$62.66^{a}\pm4.8$	$31.27^{a}\pm2.2$	$20.41^{a}\pm0.5$
	0.5	$48.00^{a}\pm6.1$	$19.73^{b}\pm0.7$	$12.58^{b}\pm0.5$
	1	$46.66^{a}\pm7.0$	$17.22^{bc}\pm0.2$	$12.05^{bc}\pm1.3$
	2	$45.33^{a}\pm8.7$	$12.83^{c}\pm1.9$	$12.00^{bc}\pm0.8$
	4	$44.00^{a}\pm2.3$	$8.28^{d}\pm0.4$	$9.54^{c}\pm1.0$

* Means with different letters in the same column are significantly different according to Duncan's test at the $p < 0.05$ significance level

On the other hand, the fruit extract of proso millet (*Panicum milliaceum*) exhibited higher allelopathic activity than the leaf extract. At the highest dose of the leaf extract, inhibition of germination, root growth, and shoot growth was determined to be 13.43%, 50.22%, and 27.53%, respectively. In applications of the fruit extract, these values reached 16.42%, 67.78%, and 60.29% (Table 5, Figure 4).

Table 5. Effect of the methanol extract of *Pistacia terebinthus* on the *Panicum milliaceum* plant

Plant part	Doses (mg/ml)	Germination (%)	Root length (mm)	Shoot length (mm)
Leaf	**Kontrol**	$89.33^{a}\pm1.3$	$52.90^{a}\pm2.7$	$12.82^{a}\pm2.4$
	0.5	$81.33^{ab}\pm2.6$	$34.92^{b}\pm6.9$	$10.83^{a}\pm1.1$
	1	$81.33^{ab}\pm3.5$	$34.84^{b}\pm3.3$	$10.45^{a}\pm3.0$
	2	$78.66^{b}\pm2.6$	$32.58^{b}\pm2.8$	$9.39^{a}\pm1.2$
	4	$77.33^{b}\pm1.3$	$26.33^{b}\pm3.3$	$9.29^{a}\pm2.5$
Fruit	**Kontrol**	$89.33^{a}\pm1.3$	$52.90^{a}\pm2.7$	$12.82^{a}\pm2.4$
	0.5	$78.66^{b}\pm3.5$	$22.98^{b}\pm3.3$	$6.71^{b}\pm2.5$
	1	$77.33^{b}\pm4.8$	$21.79^{b}\pm1.7$	$5.40^{b}\pm0.5$
	2	$76.00^{b}\pm2.3$	$19.29^{b}\pm1.5$	$5.15^{b}\pm1.5$
	4	$74.66^{b}\pm1.3$	$17.04^{b}\pm0.7$	$5.09^{b}\pm0.4$

* Means with different letters in the same column are significantly different according to Duncan's test at the $p < 0.05$ significance level

Figure 4. Effect of the methanol extract of *P. terebinthus* on the growth of *Panicum milliaceum*

Overall, when all test species were considered together, the fruit extracts of menengiç exhibited stronger allelopathic effects than the leaf extracts. The highest inhibition values were recorded as complete suppression of shoot growth in hollyhock (100%), 82.3% inhibition of root growth in garden cress, and 73.5% reduction in root growth in purslane. These findings indicate that the allelochemical compounds present particularly in the fruit tissues of menengiç possess significant biological activity capable of suppressing the early developmental stages of weeds. In particular, the marked inhibition of root and shoot growth suggests that menengiç fruits contain bioactive compounds that may be utilized for weed control.

4. DISCUSSION

In this study, the leaf and fruit extracts of *Pistacia terebinthus* L. were found to exert significant allelopathic effects on the germination and seedling growth of test plants in a dose-dependent manner. In particular, the stronger suppression of root and shoot growth by fruit extracts compared with leaf extracts indicates that the bioactive compounds present in menengiç fruits have a higher phytotoxic potential. Numerous studies have shown that menengiç species are rich in phenolic acids, flavonoids, tannins, and terpenoids (Topçu et al., 2007; Hacıbekiroglu ve ark., 2015). These compounds are known to suppress germination and seedling growth by affecting cell division, enzyme activities, and water uptake in plants (Inderjit and Duke, 2003).

In the present study, the highest inhibition values were observed in root growth. In particular, the 82.3% suppression of root growth in garden cress and the 73.5% inhibition in purslane at the 4 mg mL^{-1} dose of the fruit extract indicate that allelochemical compounds primarily affect root tissues. Similarly, studies conducted with plant extracts showing allelopathic effects have reported that root elongation is a more sensitive parameter than shoot growth and germination (Xuan et al., 2005). Because roots are in direct contact with allelochemicals, these tissues are generally considered more sensitive to phytotoxic effects (Rice, 1984).

Most studies on menengiç have focused on its antioxidant, antimicrobial, and pharmacological activities. For example, Topçu et al. (2007) reported that *P. terebinthus* extracts contain high levels of flavonoids and phenolic compounds, and that these compounds exhibit strong antioxidant activity. Similarly, Hacıbekiroglu et al. (2015) reported that menengiç extracts display significant antimicrobial activity and that the principal constituents of the extracts include gallic acid, catechin derivatives, and various terpenoids. These compounds are known to be associated with allelopathic activity in many plants (Li et al., 2010). Therefore, the phytotoxic effect observed in the present study is also likely to be attributed to these phenolic and terpenoid compounds. In a study investigating the inhibitory effects of aqueous extracts prepared from the leaves of *Pistacia vera* at concentrations of 3%, 6%, 12%, and 25% on three weed species (*Lolium rigidum*, *Solanum nigrum*, and *Lactuca serriola*) and four crop species (maize cv. Celestquatro, barley cv. Yurico, wheat cv. Rimbaud, and lentil cv. Castellana), the extracts were reported to significantly suppress germination in weeds, particularly in broad-leaved species, whereas no adverse effect on germination rates was observed in crop plants (Saludes-Zanfaño et al., 2024).

In the present study, the complete inhibition (100%) of shoot growth in hollyhock at all doses of the fruit extract at and above 1 mg mL^{-1} indicates that menengiç extracts possess a selective and strong allelopathic effect against certain species. These interspecific differences are likely due to variations in plant tolerance to allelochemicals (Inderjit and Duke, 2003). Likewise, the substantial suppression of root and shoot growth in wheat despite the relative preservation of germination rates suggests that allelochemicals primarily target early seedling development rather than germination itself.

In recent years, interest in natural bioherbicides has increased due to the environmental and ecological problems associated with the use of synthetic herbicides. The high inhibitory effects of menengiç extracts, particularly on weed species such as purslane and millet weed, indicate that this plant may be considered a potential candidate for natural weed control. Duke and Dayan (2015) noted that plant extracts rich in phenolics and terpenoids constitute an important source for the development of next-generation environmentally friendly bioherbicides. In this context, the present findings suggest that menengiç fruit extracts may represent a natural source of allelochemicals for weed management.

In conclusion, in addition to its previously reported antioxidant and antimicrobial activities, menengiç was shown to possess a marked allelopathic potential. In particular, the high inhibitory effect of fruit extracts on root and shoot growth indicates that menengiç is an important plant that may be evaluated in bioherbicide development studies. However, further phytochemical and biological studies are needed to identify the compounds responsible for this activity and to clarify their mechanisms of action.

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CHAPTER 11

RESILIENCE OF FOOD SYSTEMS, FOOD INFLATION, AND FOOD LITERACY IN TÜRKİYE

An Assessment from Global Vulnerabilities to the Consumer

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1. INTRODUCTION

Recent geopolitical developments have made agriculture and food strategic issues directly linked to national security, social welfare, and sustainable development, rather than areas that can be evaluated solely in terms of production volume or economic activity. Global disruptions, climate change, fluctuations in energy prices, water scarcity, logistical disruptions, the contraction of agricultural land, and the declining engagement of young people in agriculture are placing decisive pressure on the continuity of food supply. Therefore, agricultural policies need to be addressed not only within a conventional framework aimed at increasing production, but through a holistic approach that considers climate, energy, water, technology, the environment, and social welfare together. In the future, the capacity to produce safe and sustainable food is expected to gain a more visible place, alongside military capacity, among the factors determining countries' positions within the international system (Erdoğan, 2026a).

Global food and agricultural systems are being reshaped by population growth, changes in income levels and consumption habits, climate pressures, and geopolitical uncertainties. While food demand is expected to continue increasing, rising energy and agricultural input costs, the slowdown in productivity growth, post-harvest losses, declining biodiversity, and transboundary pests and diseases are further deepening the vulnerability of these systems. This is the main reason why the need for structural transformation has become more evident throughout the food chain, from production to processing and storage, and from logistics to consumption. Unsafe food processing practices, particularly in low-income countries, are estimated to cause at least USD 110 billion in economic losses each year (Erdoğan, 2026b).

In Turkish agriculture, climate-related risks make production planning and predictability more difficult. Water stress, extreme weather events, and plant health risks can affect not only production volumes but also costs, prices, and rural incomes. Climate, water, technology, and resource efficiency therefore need to be considered together with the resilience of the food system (Erdoğan, 2026a).

Food has long been discussed largely in terms of production volume. Yet, the physical availability of food in a country does not mean that everyone can regularly access adequate, safe, and nutritious food. Food security is a broad concept that encompasses not only food availability, but also economic and physical access, stable provision, and nutritional adequacy. Assessments concerning Türkiye also emphasize that food security is a comprehensive area extending beyond food safety to include issues such as affordability, access, and natural resources (Yücel, 2022).

This framework demonstrates that the food system consists of more than agricultural production alone. Each stage, production, processing, storage, logistics, retail, consumption, and food loss and waste has environmental, economic, and social consequences. Ak Bingül (2025) draws attention to the fact that sustainable transformation in food systems should be assessed not only through technical efficiency, but also through income equity, governance, cultural compatibility, and social equality. This is where the concept of resilience becomes important. The objective should not be merely to expand the system, but to establish a structure capable of protecting access to food, production capacity, and dietary quality in the face of climate-, price-, health-, geopolitical-, or trade-related shocks.

Türkiye's National Roadmap Towards Sustainable Food Systems addresses access to safe and nutritious food, healthy and sustainable consumption, production in harmony with nature, fair livelihoods, and resilience to shocks within the same transformation framework (Ministry of Agriculture and Forestry, 2021). This approach also makes it insufficient to assess food inflation solely through changes in the consumer price index. Food inflation should be considered together with the production structure, input costs, supply capacity, and households' access to food.

Assessing the food system within this broader framework requires moving away from considering production growth alone as a measure of success. The abundant availability of a product does not indicate that low-income households can access it regularly; likewise, the functioning of the supply chain does not necessarily mean that the environmental costs of production are at an acceptable level. In Türkiye's country report, the Ministry of Agriculture and Forestry (2021), referring to the Food and Agriculture Organization of the United Nations (FAO), defines the food system as the set

of relationships among the actors involved in the production, collection, distribution, consumption, and disposal of food products in agriculture, forestry, and fisheries, the related value-added activities, and the relevant components of the broader economic, social, and natural environment. This definition requires that food inflation not be considered independently of production costs, nor food literacy independently of individual choices alone.

Global indicators clearly demonstrate the significance of this relationship. According to the joint assessment of FAO, IFAD, UNICEF, WFP, and WHO (2025), approximately 673 million people faced hunger in 2024, around 2.3 billion people experienced moderate or severe food insecurity, and 2.60 billion people could not afford a healthy diet. These data show that the availability of food in the market alone is insufficient and that purchasing power and nutritional quality should also be regarded as key outcomes of the food system. Food security and food safety are closely related but distinct concepts. The food security refers to the availability, accessibility, and adequate nutritional value of food. The food safety, on the other hand, concerns ensuring that foods do not pose risks to human health. Unsafe foods can cause not only acute poisoning but may also affect various systems in the body over the long term, creating the basis for chronic health problems. Therefore, food safety is an issue that concerns not only the health of today's population but also that of future generations. To draw attention to the importance of this field, the United Nations General Assembly declared 7 June as "World Food Safety Day" through a resolution adopted on 20 December 2018 (Erdoğan, 2026b).

For a healthy life, everyone has the right to sustainable access to adequate, safe, and healthy food. Today, approximately 2 billion people worldwide lack food security, while around 673 million people are unable to access sufficient food regularly. In addition, nearly 3 billion people, representing 40 percent of the world's population, are reported to be unable to access a healthy diet. Each year, approximately 600 million people fall ill due to the consumption of unsafe food, and around half a million people lose their lives. These indicators clearly demonstrate the importance of food safety for public health. It is not possible to speak fully of the food safety in an environment where food security is not ensured, nor of the food security where the food safety has not been established (Erdoğan, 2026b). In this chapter, resilience is addressed not as a static characteristic that protects the system from

every kind of change, but as the capacity to maintain essential functions in the face of shocks, adapt when necessary, and move towards more appropriate outcomes. Such an approach acknowledges that the risks arising at every link of the chain from production to consumption have consequences for social welfare, public health, and environmental sustainability. Figure 1 illustrates the relationship between the risks arising within food systems and the resilience capacity that helps limit their effects.

Climate change and water stress, geopolitical uncertainties, energy and input costs, and disruptions in supply chains often do not occur independently; rather, they reinforce one another.

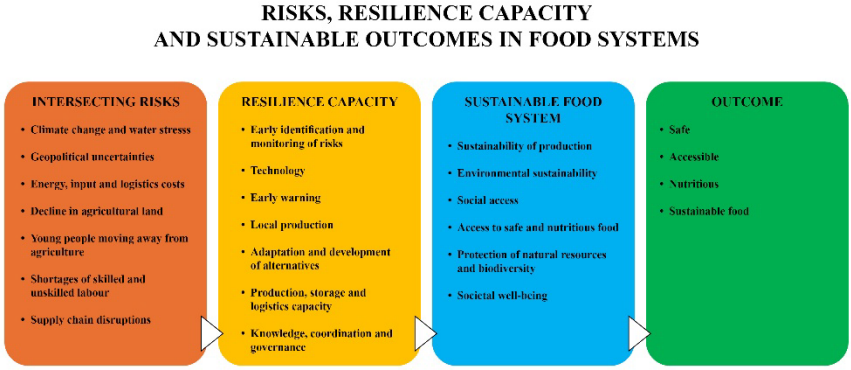

Figure 1. Relationship between risks, resilience capacity, and sustainable outcomes in food systems

In this context, resilience is not limited to responding after a crisis has occurred. It also entails the capacity to anticipate risks, adapt to changing conditions, maintain production and supply capacity, and establish coordination based on reliable information. Strengthening this capacity contributes to the continuity of production, the protection of access to safe and nutritious food, the reduction of pressure on natural resources, and the support of social welfare.

2. GLOBAL PRESSURES THAT MAKE FOOD SYSTEMS VULNERABLE

2.1.Supply Chains, Geopolitical Uncertainty, and the Cost Channel

The pandemic demonstrated that global food chains depend not only on production but also on labour mobility, transportation, energy, and predictable trade flows. FAO assessments show that lockdowns sharply affected demand and oil prices, while logistical constraints and sudden changes in market conditions placed pressure on food supply chains (FAO, 2020). Since the post-pandemic recovery did not occur simultaneously, supply pressures and cost increases continued for many products.

Geopolitical tensions have made this vulnerability more visible. The dominance of certain regions in grain, oilseed, energy, and fertiliser markets means that disruptions in trade routes can affect food supply and costs at the same time. An OECD study published in October 2025, assessing data from August 2025, reported that food price levels in OECD countries were 45.8 percent higher than in December 2019, while the cumulative increase in Türkiye over the same period exceeded 790 percent (OECD, 2025). This comparison shows that global shocks do not produce outcomes of the same magnitude in every country and that national economic and sectoral structures are decisive.

Food price movements in recent years demonstrate how these multiple pressures interact. FAO, IFAD, UNICEF, WFP, and WHO (2025) state that food price inflation remained above global headline inflation in the period after 2020 and that high food prices aggravated food insecurity, particularly in low-income countries. Therefore, cost increases arising from energy, fertiliser, freight, or exchange rates do not appear merely as shelf prices at the end of the value chain, they also affect the quality of household meals and dietary diversity.

Movements in global prices and food inflation in Türkiye do not occur in the same direction or with the same intensity. The FAO Food Price Index rose to 131.1 points in July 2026, increasing by 0.6 percent from the previous month and by 1.0 percent compared with the same month of the previous year. The monthly 3.4 percent rise in cereal prices and the increase in vegetable oil

prices indicate that risks in global markets have not disappeared entirely. In contrast, the higher annual increase in food prices in Türkiye suggests that global price movements are reflected differently in domestic prices when combined with exchange rates, dependence on imported inputs, financing conditions, and value-chain problems (FAO, 2026; TÜİK, 2026b).

Supply chain resilience is therefore not merely a matter of finding alternative transport routes. Making dependencies in critical inputs and products visible, strengthening storage and cold-chain capacity, improving the transparency of market information, and protecting small producers' access to markets should be addressed together. Increasing local capacity is important. However, this does not mean establishing a closed structure that excludes trade. The essential issue is to know which dependency creates risk at which stage and to develop realistic options against that risk.

2.2. Climate Change, Water Pressure, and Production Risk

Climate change is both a direct production risk and an issue of cost and access for food systems. Rising temperatures, droughts, floods, fires, and unseasonal weather events place pressure on yields, product quality, and production planning, while their effects on water availability and energy costs extend this pressure to other links in the value chain. FAO (2015) emphasizes that climate change affects food security through production, natural resources, and rural livelihoods.

Empirical findings for Türkiye also indicate that climate risk cannot be considered separately from price stability. In their analysis for the 1990–2021 period, Kömürcüoğlu and Artan (2024) showed that climate change creates long-term inflationary pressure on food and consumer inflation and that there is bidirectional causality among the variables. Günsan and Yergin (2025) also state that the pressure created by climate change on agricultural production and food prices is one of the important components of the debate on food inflation in Türkiye. Therefore, climate adaptation is not only environmental policy; it is also food-access and price-stability policy.

The effect of climate change on food prices extends beyond harvest losses. Water scarcity, extreme temperatures, disease and pest pressure can create costs throughout the value chain by affecting product quality, harvest timing, storage losses, and logistics planning. Therefore, climate adaptation is

not only part of environmental policy but also part of policies for the continuity of production and price stability. The assessment by Erdoğan et al. (2024) in the context of the European Green Deal emphasizes that climate change, biodiversity, and the circular economy should be considered in order for Türkiye's agricultural sector to sustain its competitiveness.

The practical reflection of adaptation policies in the field lies in the joint implementation of tools such as practices that protect soil productivity, efficient water use, production patterns suited to climate risk, early-warning infrastructure, and integrated pest management. When addressed individually, these tools may have limited effects; however, when supported by producers' access to information, finance, and technical advice, they can reduce the likelihood of risk turning into damage. In this respect, climate adaptation is not only a long-term environmental objective but also an issue related to the quality of everyday production decisions.

2.3. Intersecting Shocks and the Cumulative Nature of Vulnerability

An important characteristic of risks in food systems is that they often do not emerge in isolation. Drought may weaken production while increasing energy costs; high energy costs can place pressure on irrigation and the cold chain, while logistical disruptions may make it more difficult to deliver products in the field or in storage to consumers. If exchange-rate movements raise the cost of imported inputs during the same period, these effects reinforce one another. Resilience analysis therefore requires understanding the channels through which shocks amplify one another rather than simply listing them individually. Zurek et al. (2022) draw attention to the complex and uncertain feedbacks and trade-offs among food-system outcomes. This framework shows that decisions in agriculture, energy, trade, health, and the environment should not be addressed independently of one another.

This cumulative effect is particularly visible for small- and medium-sized producers. When producers face drought, price uncertainty, labour problems, and financing costs simultaneously, a support mechanism designed for a single risk may not be sufficient. In this respect, the emphasis in the country report of the Ministry of Agriculture and Forestry (2021) on agricultural insurance, extension and advisory services, access to finance for

small producers and enterprises, and support for vulnerable groups is meaningful. Resilience does not mean merely supporting producers after a crisis, it means enabling them to build the capacity to expand their options before the crisis occurs.

Recognising the links between shocks also increases the need for coordination among data and institutions. Managing meteorological early warning and water management, plant health monitoring systems and production planning, and market information and storage capacity separately can delay decision-making. In contrast, regular data sharing among these areas enables regional risks to become visible earlier and makes it possible to intervene not only after damage has occurred but also during the preparation stage.

2.4. Geopolitical Risks and the Vulnerability of Food Systems

Geopolitical developments clearly demonstrate the extent to which food systems depend not only on production conditions but also on energy, fertilisers, transport corridors, and international trade. The Russia–Ukraine war has had significant consequences for global markets because of disruptions in grain and oilseed trade through the Black Sea. The Russian Federation and Ukraine are among the major suppliers to world markets for wheat, maize, barley, and sunflower oil. The Russian Federation is also one of the leading exporters of nitrogen fertilisers and a major supplier of phosphorus and potassium fertilisers (FAO, 2022). Therefore, the effects of the war have not been limited to product supply, they have also placed pressure on input costs, transport opportunities, and access to food, particularly in countries dependent on imports. Indeed, this pressure also led to the relaxation of environmentally oriented agricultural policies at the global level. Following the Russia–Ukraine war, the European Union temporarily eased the non-productive land requirement for biodiversity under the Common Agricultural Policy (GAEC 8) and allowed these lands to be brought into production in order to secure food supply (European Commission, 2024).

In this process, the Grain Corridor Agreement should be regarded as an important achievement demonstrating Türkiye's agricultural diplomacy and mediation capacity. In response to the global grain crisis that emerged after the Russia–Ukraine war, Türkiye, together with the United Nations, assumed a role

in coordinating the initiative and contributed to the safe delivery of millions of tonnes of grain waiting in Ukrainian ports to world markets. The Grain Corridor is important not only for maintaining food supply and easing pressure on international markets, but also for demonstrating the role of agricultural diplomacy in food security and the preservation of peace (Erdoğan et al., 2025).

The humanitarian dimension in the history of Türkiye's agricultural diplomacy is also noteworthy in this context. As reported by Erdoğan et al. (2025), in-kind and monetary assistance provided during the Irish Famine of 1848 are among the historical examples of Türkiye's solidaristic approach to food crises. The Irish Potato Famine also demonstrates that food crises cannot be explained solely by losses in crop production, they need to be assessed together with dependence on a single crop in the production structure, poverty, inequalities in access to food, and the policy choices of the period. This historical experience reveals the importance of production diversity, plant health management, economic access, and public intervention capacity in food systems (Ó Gráda, 2009).

The chokepoints through which energy and fertiliser trade passes also increase agricultural production's exposure to geopolitical risks. A disruption in energy and maritime transport through the Strait of Hormuz may directly affect fertiliser markets, as well as oil, natural gas, and derivative products. According to the assessment of the International Energy Agency, approximately one quarter of global seaborne oil trade passes through the Strait of Hormuz. The same route is also critically important for more than 30 percent of global urea trade and for approximately one fifth of ammonia and phosphate trade (International Energy Agency [IEA], 2026). Such disruptions can create new pressures throughout the chain from production to consumer prices by increasing energy and fertiliser costs used in agricultural production.

These developments demonstrate that the resilience of food systems cannot be explained solely by agricultural production capacity. Strengthening production and storage capacity for strategic products, reducing dependence on external sources for agricultural inputs, developing alternative supply and logistics routes, expanding early-warning systems, and supporting climate-adapted production models are important for limiting the effects of geopolitical shocks on food systems.

3. RESILIENCE APPROACH IN FOOD SYSTEMS

Resilience does not mean that a system remains completely unaffected by a shock. What matters is its capacity to limit the impact of the shock, adapt to changing conditions, and recover while maintaining its essential functions. In the context of the food system, these functions include sustaining production, maintaining supply flows, reducing food loss, ensuring continued access to safe and nutritious food, and preventing the weakening of producers' livelihood capacity. Therefore, resilience is concerned not only with production volume, but also with diversity, local capacity, logistical adequacy, and the quality of decision-making processes.

Strengthening local production capacity and diversifying supply chains reduce the risk arising from dependence on a single source or transport route. However, a local approach does not imply a closed-market understanding; what is critical is making dependencies in input and product flows visible, developing alternatives, and using resources more efficiently. In this respect, the National Roadmap's simultaneous establishment of objectives related to production in harmony with nature, fair livelihoods, and resilience to shocks is valuable (Ministry of Agriculture and Forestry, 2021).

Food loss and waste are also central to the discussion of resilience. A product that is lost or wasted does not merely lose its economic value, the land, water, energy, labour, and logistical capacity used in its production are also expended without return. Ak Bingül (2025) states that circular practices, such as the redistribution of surplus products in retail and the marketing of imperfect products, can contribute to resource efficiency. Therefore, reducing loss and waste is one of the fundamental resilience tools that supports the more effective use of resources in food systems.

3.1. The Boundaries of Resilience

Before using the concept of resilience, it is necessary to determine which outcome is intended to be protected. Zurek et al. (2022) draw attention to four questions regarding food-system resilience: What is to be made more resilient? Against which shock or stress is resilience to be strengthened? From whose perspective is this assessment being made? What time horizon is being used? Although these questions may appear simple, they are decisive for policy design. For example, uninterrupted product flows for a supplier, income

continuity for a small producer, and consumers' access to nutritious food may not benefit to the same extent from the same intervention.

The same study identifies three different approaches to resilience: robustness, recovery, and reorientation. Robustness refers to the diversity and protective capacity that reduce the impact of a shock; recovery refers to the return to essential functions after a disruption; and reorientation refers to the transition towards more appropriate outcomes when the existing order is not environmentally or socially sustainable (Zurek et al., 2022). Making this distinction in discussions concerning the food system in Türkiye prevents the conflation of measures intended to alleviate short-term price pressure with policies designed to ensure long-term transformation.

From this perspective, the indicators of resilience also become more diverse. Alongside production volume, the condition of water and soil resources, producers' income stability, the continuity of input supply, the level of loss and waste, cold-chain adequacy, the transparency of information in the market, and consumers' access to nutritious food should be monitored together. This is why the simultaneous consideration of economic, social, and environmental dimensions in the national roadmap of the Ministry of Agriculture and Forestry (2021) is important.

3.2. The Place of Loss and Waste within Resilience

Food loss and waste are often associated only with household consumption habits. However, the causes of losses at the stages of harvesting, grading, storage, transport, retail, and household consumption differ. With every product lost, water, soil, energy, labour, and logistical capacity are also used without return. Reducing loss and waste is therefore a resilience strategy that both improves resource efficiency and supports access to food. The solution should not be placed solely on consumers; it also needs to include storage, cold-chain infrastructure, packaging, market standards, and redistribution mechanisms.

3.3. Resilience, Sustainability, and Trade-offs

Although resilience and sustainability are often used interchangeably, the two concepts do not fully overlap. A system may be resilient to a short-term disruption, but if it achieves this through high water use, intensive energy consumption, or a market structure that excludes certain producers, it is not

sustainable in the long term. Zurek et al. (2022) draw attention to the need to assess trade-offs among food-system outcomes explicitly. Therefore, the balance established between increased production, price accessibility, environmental impact, and income distribution shows that resilience involves a normative choice.

Türkiye's approach to sustainable food systems also requires the simultaneous consideration of economic, social, and environmental dimensions (Ministry of Agriculture and Forestry, 2021). An economically viable production structure should take into account not only the continuity of producer income but also consumer access. Socially, the position of different groups in the food system, including women, young people, seasonal workers, and low-income households, is important. Environmentally, an assessment of efficiency remains incomplete unless the relationships among water, soil, biodiversity, and food loss and waste are taken into account.

Policy objectives therefore need to be clearly prioritised. It may not always be possible to maximise all outcomes simultaneously. However, it is possible to discuss in advance which trade-offs are acceptable, which thresholds should not be exceeded, and on which actors the costs are concentrated. The real contribution of the concept of resilience lies precisely in carrying this discussion beyond production volume.

4. STRUCTURAL DYNAMICS OF FOOD INFLATION IN TÜRKİYE

4.1. Exchange Rates, Imported Inputs, and Price Inertia

Annual inflation, as measured by the Consumer Price Index (CPI), reached 3.4 percent in February 2026, compared with 3.3 percent in January 2026 in OECD countries, and remained generally stable. This figure represented the largest increase of 0.8 percentage points compared with January 2026 in Türkiye. Food inflation in OECD countries rose from 3.7 percent in January 2026 to 4 percent in February 2026. This increase was largely driven by the 4.7 percent rise in Türkiye (OECD, 2026).

Although the food inflation in Türkiye is affected by global price movements, these effects are amplified through country-specific structural channels. Dependence on external sources for fertilisers, energy, feed, plant protection products, machinery and equipment, and certain raw materials

causes changes in exchange rates to be transmitted to production costs and then to consumer prices.

Examining food price formation, Schroeder et al. (2018) analysed the key inefficiencies in Türkiye's agricultural sector and discussed the policy actions necessary to enhance the sector's competitiveness. The results of the analysis showed that inefficiencies in agricultural markets, supported by macroeconomic factors, had placed food prices on an upward trajectory. The research findings indicated the need to move away from short-term policy responses towards a broader policy strategy aimed at stabilising prices through increased productivity and improved market linkages.

The macroeconomic dimension of the food inflation in Türkiye can be more clearly observed through the relationship between exchange rates and input costs. Using a Structural VAR (SVAR) model covering the period 2008:8–2020:8, Şahin Kutlu (2021) showed that the food price index responded statistically significantly to exchange-rate shocks among the variables included in the model. This finding does not mean that the exchange rate is the sole determinant of food prices, however, it demonstrates that it constitutes a structural channel due to dependence on imported inputs and cost pass-through.

Nevertheless, food prices do not respond only to current changes in costs. The findings of Yıldırım (2021) show that there is strong inertia in food prices and that producer prices, interest rates, money supply, and exchange rates are effective in explaining price movements. This suggests that past price increases can be carried over into subsequent periods through expectations and pricing behaviour.

Using a structural VAR model for Türkiye, Oral et al. (2023) examined the relationship between food prices and global and macroeconomic variables such as exchange rates, the global fertiliser price index, oil prices, world food prices, and money supply. The findings show that the exchange rate is one of the prominent factors explaining changes in food prices.

The effects of possible factors determining food inflation were analysed by Bozkurt and Mutlu Çamoğlu (2025) using a Bootstrap ARDL model with a Fourier function. According to the research findings, the existence of cointegration among the food consumer price index, food producer price index, food industry production index, US dollar exchange rate, and policy interest rate variables was accepted. The short- and long-term findings of the analysis

showed that increases in the food products producer price index and the exchange rate had strong inflationary effects on food inflation.

Serttaş and Türk (2025) point out that dependence on external sources for agricultural inputs and exchange-rate movements create a mutually reinforcing cost-price spiral. Within this framework, 70–90 percent of fertiliser raw materials and approximately 60 percent of animal feed raw materials are imported. In this structure, changes in the exchange rate are reflected first in input costs, then in producer prices, and finally in consumer prices. Therefore, it has been emphasized that it is not sufficient to explain food inflation only through demand conditions and that exchange rates, financing, input supply, and production costs need to be evaluated together.

4.2. Seasonality, Supply Inefficiency, and Price Transmission

In Türkiye, adverse climatic events create risks for the general price level, particularly for food, by causing short-term supply shocks. Within this framework, the Central Bank of the Republic of Türkiye (2024) examined the relationship between "climate change" and "food inflation" in Türkiye using several indicators. The study concluded that climate change would increase global food costs by an average of between 1.5 and 1.8 percentage points each year until 2035. These analyses also indicated that the seasonal structure of fresh fruit and vegetable prices has changed and emphasized that climate change plays an important role in this development.

The biological production cycle of agriculture causes food prices to be susceptible to seasonal fluctuations. However, "seasonality" alone cannot explain these fluctuations. Disruptions related to storage, the cold chain, transportation, wholesale trade, retail, and market information can widen the gap between the price received by producers and the price paid by consumers. The World Bank (2020) assesses that low productivity, local supply-demand imbalances, seasonality, and structural inefficiencies are decisive factors in food price inflation in Türkiye.

This transmission problem also affects production decisions. If high prices observed by consumers are not reflected in producer income to the same extent, producers' ability to invest in productivity-enhancing measures weakens. Thus, price pressure in the short term may turn into a cycle that limits supply capacity in the medium term. Yücel (2022) emphasizes that, in

combating food inflation, investment in technology, logistics, and supply-side areas along the value chain is as important as stabilising the exchange rate.

The transmission of food prices from producers to consumers is also not homogeneous. In fresh fruit and vegetables, the harvest period, storage opportunities, product perishability, and transport conditions have different weights, while in animal products, feed, energy, and processing costs are more significant. Therefore, food inflation should not be assessed as though it reflects a single market behaviour; differences at the level of product group, region, and value chain should be taken into account. Regular monitoring that makes visible the link in the supply chain where cost or margin increases are concentrated is necessary for both competition and supply planning.

Vulnerability in the food system does not arise only from costs and price transmission in the domestic market; concentration in export markets is also an important area of risk. Serttaş and Türk (2025) draw attention to the high share of the Iraqi market, particularly in flour and poultry meat exports. Dependence on one or a limited number of markets for certain products creates additional vulnerability for producers and exporters in the face of customs tariffs, the price advantages of competitor countries, or changes in import policies. Therefore, resilience should include not only production and logistics capacity but also the diversification of markets and trade routes.

4.3. Social Impact and Economic Access

The distributional effect of rising food prices is not equal. The share of food expenditure within the household budget is higher for low-income households. The same price increase therefore has more severe consequences for these groups' dietary diversity and access to food.

Yıldırım (2021) emphasizes that food consumption occupies an important place among essential expenditures in developing economies, especially for low-income households, and that movements in food prices are important for overall price stability. A resilient food system should consequently be considered as a system that protects not only supply but also economic access.

4.4. Interpreting Current Official Indicators

Several indicators have been used to emphasise the importance of the issue from a global perspective. Within this framework, Table 4.1 presents the FAO Food Price Index and Cereal Price Index, as well as the all-food CPI data and prices for selected food items prepared by the Economic Research Service (ERS) of the United States Department of Agriculture (USDA).

Table 4.1. Global Food Prices and Selected Food Price Indicators in the United States (June–July 2026)

Indicator	Value
FAO Food Price Index (points)	131,1
FAO Food Price Index (monthly change, percent)	0,6
FAO Cereal Price Index (monthly change, percent)	3,4
US all-food CPI (annual change, percent)	3,0
US beef and veal prices (annual change, percent)	11,8
US retail egg prices (annual change, percent)	-27,9

Source: FAO (2026); USDA ERS (2026).

The FAO Food Price Index averaged 131.1 points in July 2026. This value was 0.6 percent higher than the June 2026 level and 1 percent higher than the previous year. The FAO Cereal Price Index reversed its decline in May 2026, increasing by 3.4 percent compared with June 2026 and standing 6.9 percent above its July 2025 level. Global wheat prices increased by 5.8 percent amid continuing disruptions in Black Sea export flows and growing concerns about the possible impact of recent heatwaves on crop yields in some major producing countries (FAO, 2026).

US beef and veal prices increased by 1.4 percent from May 2026 to June 2026 and were 11.8 percent higher in June 2026 than in June 2025. According to the USDA ERS Livestock, Dairy, and Poultry Outlook report, the US cattle herd declined to its lowest level in 75 years, and wholesale beef prices were assessed as remaining at all-time highs for this time of year. US egg prices, on the other hand, declined by 0.8 percent from May 2026 to June 2026 and were 27.9 percent lower in June 2026 than in June 2025. Among the reasons for this decline is the Highly Pathogenic Avian Influenza (HPAI) outbreak, which began in 2022 and is still ongoing, causing egg prices to rise by reducing egg yield and egg production (USDA ERS, 2026).

TÜİK's July 2026 data show that the annual rate of change in the Consumer Price Index (CPI) was 31.8 percent. The CPI increased by 37.5 percent annually in the main expenditure group of "food and non-alcoholic beverages," and this group's contribution to the annual change was 8.9 percent (TÜİK, 2026b).

The Agricultural Input Price Index (Agricultural IPI) monitors price movements in goods and services used in agricultural production, as well as investment inputs. The annual change trend of the Agricultural IPI for the 2020–2026 period is presented in Table 4.2.

Table 4.3. Annual Rates of Change in Agricultural IPI by Subgroup (percent, May 2026)

Year	2020	2021	2022	2023	2024	2025	2026
Percentage	17,5	64,4	144,8	73,6	52,2	34,6	36,7*

Source: Turkish Statistical Institute, Agricultural Input Price Index (TÜİK, 2026a).
* May 2026 data.

TÜİK's May 2026 data show that the Agricultural IPI increased by 36.7 percent annually (TÜİK, 2026a). The rates of change in Agricultural IPI subgroups are presented in Table 4.3. As can be seen from Table 4.3, the subgroup with the highest annual change in the Agricultural IPI was "Fertilisers and Soil Improvers," with an increase of 63.6 percent.

Table 4.3. Annual Rates of Change in Agricultural IPI by Subgroup (percent, May 2026)

Subgroup	Percentage
Fertilisers and Soil Improvers	63,6
Energy and Lubricants	40,8
Veterinary Expenses	36,0
Animal Feed	35,5
Seeds and Planting Material	34,9
Other Goods and Services	34,7
Buildings	28,9
Building Maintenance Expenses	26,5
Machinery Maintenance Expenses	25,8
Agricultural Chemicals	22,7
Materials	20,5

Source: Turkish Statistical Institute, Agricultural Input Price Index (TÜİK, 2026a).

5. GLOBAL PRESSURES THAT MAKE FOOD SYSTEMS VULNERABLE

5.1. Supply Chains, Geopolitical Uncertainty, and the Cost Channel

The pandemic demonstrated that global food chains depend not only on production but also on labour mobility, transportation, energy, and predictable trade flows. FAO assessments show that lockdowns sharply affected demand and oil prices, while logistical constraints and sudden changes in market conditions placed pressure on food supply chains (FAO, 2020). Since the post-pandemic recovery did not occur simultaneously, supply pressures and cost increases continued for many products.

Geopolitical tensions have made this vulnerability more visible. The dominance of certain regions in grain, oilseed, energy, and fertiliser markets means that disruptions in trade routes can affect food supply and costs at the same time. An OECD study published in October 2025, assessing data from August 2025, reported that food price levels in OECD countries were 45.8 percent higher than in December 2019, while the cumulative increase in Türkiye over the same period exceeded 790 percent (OECD, 2025). This comparison shows that global shocks do not produce outcomes of the same magnitude in every country and that national economic and sectoral structures are decisive.

Food price movements in recent years demonstrate how these multiple pressures interact. FAO, IFAD, UNICEF, WFP, and WHO (2025) state that food price inflation remained above global headline inflation in the period after 2020 and that high food prices aggravated food insecurity, particularly in low-income countries. Therefore, cost increases arising from energy, fertiliser, freight, or exchange rates do not appear merely as shelf prices at the end of the value chain; they also affect the quality of household meals and dietary diversity.

Movements in global prices and food inflation in Türkiye do not occur in the same direction or with the same intensity. The FAO Food Price Index rose to 131.1 points in July 2026, increasing by 0.6 percent from the previous month and by 1.0 percent compared with the same month of the previous year. The monthly 3.4 percent rise in cereal prices and the increase in vegetable oil

prices indicate that risks in global markets have not disappeared entirely. In contrast, the higher annual increase in food prices in Türkiye suggests that global price movements are reflected differently in domestic prices when combined with exchange rates, dependence on imported inputs, financing conditions, and value-chain problems (FAO, 2026; TÜİK, 2026b).

Supply-chain resilience is therefore not merely a matter of finding alternative transport routes. Making dependencies in critical inputs and products visible, strengthening storage and cold-chain capacity, improving the transparency of market information, and protecting small producers' access to markets should be addressed together. Increasing local capacity is important. However, this does not mean establishing a closed structure that excludes trade. The essential issue is to know which dependency creates risk at which stage and to develop realistic options against that risk.

5.2. Climate Change, Water Pressure, and Production Risk

Climate change is both a direct production risk and an issue of cost and access for food systems. Rising temperatures, droughts, floods, fires, and unseasonal weather events place pressure on yields, product quality, and production planning, while their effects on water availability and energy costs extend this pressure to other links in the value chain. FAO (2015) emphasizes that climate change affects food security through production, natural resources, and rural livelihoods.

Empirical findings for Türkiye also indicate that climate risk cannot be considered separately from price stability. In their analysis for the 1990–2021 period, Kömürcüoğlu and Artan (2024) showed that climate change creates long-term inflationary pressure on food and consumer inflation and that there is bidirectional causality among the variables. Günsan and Yergin (2025) also state that the pressure created by climate change on agricultural production and food prices is one of the important components of the debate on food inflation in Türkiye. Therefore, climate adaptation is not only environmental policy; it is also food-access and price-stability policy.

The effect of climate change on food prices extends beyond harvest losses. Water scarcity, extreme temperatures, disease and pest pressure can create costs throughout the value chain by affecting product quality, harvest timing, storage losses, and logistics planning. Therefore, climate adaptation is

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The practical reflection of adaptation policies in the field lies in the joint implementation of tools such as practices that protect soil productivity, efficient water use, production patterns suited to climate risk, early-warning infrastructure, and integrated pest management. When addressed individually, these tools may have limited effects; however, when supported by producers' access to information, finance, and technical advice, they can reduce the likelihood of risk turning into damage. In this respect, climate adaptation is not only a long-term environmental objective but also an issue related to the quality of everyday production decisions.

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These developments demonstrate that the resilience of food systems cannot be explained solely by agricultural production capacity. Strengthening production and storage capacity for strategic products, reducing dependence on external sources for agricultural inputs, developing alternative supply and logistics routes, expanding early-warning systems, and supporting climate-adapted production models are important for limiting the effects of geopolitical shocks on food systems.

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Before using the concept of resilience, it is necessary to determine which outcome is intended to be protected. Zurek et al. (2022) draw attention to four questions regarding food-system resilience: What is to be made more resilient? Against which shock or stress is resilience to be strengthened? From whose perspective is this assessment being made? What time horizon is being used? Although these questions may appear simple, they are decisive for policy design. For example, uninterrupted product flows for a supplier, income

continuity for a small producer, and consumers' access to nutritious food may not benefit to the same extent from the same intervention.

The same study identifies three different approaches to resilience: robustness, recovery, and reorientation. Robustness refers to the diversity and protective capacity that reduce the impact of a shock; recovery refers to the return to essential functions after a disruption; and reorientation refers to the transition towards more appropriate outcomes when the existing order is not environmentally or socially sustainable (Zurek et al., 2022). Making this distinction in discussions concerning the food system in Türkiye prevents the conflation of measures intended to alleviate short-term price pressure with policies designed to ensure long-term transformation.

From this perspective, the indicators of resilience also become more diverse. Alongside production volume, the condition of water and soil resources, producers' income stability, the continuity of input supply, the level of loss and waste, cold-chain adequacy, the transparency of information in the market, and consumers' access to nutritious food should be monitored together. This is why the simultaneous consideration of economic, social, and environmental dimensions in the national roadmap of the Ministry of Agriculture and Forestry (2021) is important.

6.2. The Place of Loss and Waste within Resilience

Food loss and waste are often associated only with consumption habits at the household level. However, the causes of losses at the stages of harvesting, grading, storage, transport, retail, and household consumption differ. With every product lost, water, soil, energy, labour, and logistical capacity are also used without return. For this reason, reducing loss and waste is a resilience strategy that both improves resource efficiency and supports the accessibility of the food. The solution should not be placed solely on consumers; it also needs to include storage, cold-chain infrastructure, packaging, market standards, and redistribution mechanisms.

6.3. Resilience, Sustainability, and Trade-offs

Although resilience and sustainability are often used interchangeably, the two concepts do not fully overlap. A system may be resilient to a short-term disruption, but if it achieves this through high water use, intensive energy consumption, or a market structure that excludes certain producers, it is not

sustainable in the long term. Zurek et al. (2022) draw attention to the need to assess trade-offs among food-system outcomes explicitly. Therefore, the balance established between increased production, price accessibility, environmental impact, and income distribution shows that resilience involves a normative choice.

Türkiye's approach to sustainable food systems also requires the simultaneous consideration of economic, social, and environmental dimensions (Ministry of Agriculture and Forestry, 2021). An economically viable production structure should take into account not only the continuity of producer income but also consumer access. Socially, the position of different groups in the food system, including women, young people, seasonal workers, and low-income households, is important. Environmentally, an assessment of efficiency remains incomplete unless the relationships among water, soil, biodiversity, and food loss and waste are taken into account.

For this reason, policy objectives need to be clearly prioritised. It may not always be possible to maximise all outcomes simultaneously. However, it is possible to discuss in advance which trade-offs are acceptable, which thresholds should not be exceeded, and on which actors the costs are concentrated. The real contribution of the concept of resilience lies precisely in carrying this discussion beyond production volume.

7. NEW TOOLS FOR RESILIENT FOOD SYSTEMS

Digital agriculture, precision production, early-warning, and traceability tools are not technologies that can solve every problem on their own. However, when combined with sound governance and extension capacity, they can help producers identify risks earlier, use resources more efficiently, and make decisions along the supply chain more visible. Data-based decision-making in water, fertiliser, energy, and plant protection practices is important for managing production costs under climate pressure.

Digital traceability is functional not only for food safety, but also for monitoring food loss and waste, batch-based planning, strengthening market information, and supporting consumer confidence. However, digitalisation carried out without considering access to technology, data ownership, and the participation of small producers may create new inequalities. As Ak Bingül (2025) emphasises, technological innovations have the potential to improve

environmental efficiency, but they should also be assessed in terms of economic access and social equality.

Climate-adapted production is a field of practice that makes resource efficiency tangible. Water management, planning production patterns according to climate risk, protecting soil health, biotechnological solutions, and early-warning systems can support the continuity of production capacity. The findings of Kömürcüoğlu and Artan (2024) on the relationship between climate risks and price stability show that these tools cannot be considered independently of macroeconomic effects.

7.1. Digitalisation, Early Warning, and Extension Capacity

Digital agriculture and precision production practices are often grouped under the heading of technology in discussions of resilience. However, the value of these tools should be measured by their contribution to producers' decisions. Remote sensing, sensors, Internet of Things-based irrigation, product monitoring, and early-warning systems can make water and fertiliser use more targeted, make disease and pest risks visible earlier, and strengthen production planning. Erdoğan and Karasungur (2025) state that next-generation agricultural practices offer significant opportunities for the effective use of resources, early identification of risks, and quality monitoring.

However, the spread of technology does not automatically produce inclusive outcomes. When small producers do not have access to finance, technical support, digital skills, maintenance and repair services, and reliable data infrastructure, the technology gap may deepen inequalities in the production structure. Therefore, extension services should focus not only on introducing equipment, but also on enabling producers to interpret data, adapt applications to their own production conditions, and assess costs and benefits.

Research findings presented in TTGV Technology Perspective show how this inequality is reflected in the field. While 62 percent of producers stated that they use next-generation irrigation systems in their enterprises, this rate was 26 percent for precision agriculture applications, 7 percent for digital agriculture, and 5.3 percent for data analysis. The fact that 59.2 percent of participants need grant or loan support, 53 percent point to high interest rates, and approximately one-third highlight the lack of technical advice and information shows that technology policies cannot be limited to equipment

support alone. When financing, extension, and technical advice are not designed together, it becomes difficult for digital transformation to reach small producers (Demir, 2026).

From this perspective, traceability is not merely a control tool. Reliable maintenance of batch-based records accelerates recall processes when necessary, helps identify the stages at which food loss and waste are concentrated, and strengthens market information. The blockchain-based infrastructure and end-to-end digital traceability solutions included in the report of the Deutsche Gesellschaft für Internationale Zusammenarbeit [GIZ] (2020) offer significant potential for strengthening transparency and data reliability in the food chain. However, it is necessary to ensure that the system does not become an additional bureaucratic burden for small producers and that rights and responsibilities in data sharing are clear. In terms of resilience, what matters is not simply collecting data, but transforming it into timely and actionable decisions.

7.2. The Intersection of Food Safety and Resilience

The relationship between food safety and food security is another fundamental dimension of the resilience discussion. The availability of an unsafe product on the market cannot be regarded as a positive outcome in terms of food security; likewise, the public health objective remains incomplete when economic access to safe food is not ensured. Therefore, good agricultural practices, hygiene, storage, transport, traceability, and risk-based controls are important not only for exports or regulatory compliance, but also for safeguarding the reliability of the food system and society's capacity to withstand crises.

The food safety system in Türkiye is largely based on the "farm-to-fork" approach. This approach refers to the implementation of controls and inspections throughout the entire process from the production stage of food until it reaches the consumer. According to Ministry of Agriculture and Forestry data, approximately 7,200 food control officers are employed in Türkiye, and a total of 138 food control laboratories, 41 public and 97 private, are in operation. Thanks to this infrastructure, more than 1.3 million food inspections are conducted each year. The legal regulations concerning food safety in Türkiye date back to the early years of the Republic; one of the first

comprehensive regulations in this field was the General Public Health Law of 1930. Today, the food safety system is largely implemented within the framework of Law No. 5996 on Veterinary Services, Plant Health, Food and Feed (Erdoğan, 2026b).

Food safety policy in the European Union is likewise shaped around the "farm-to-fork" approach. The aim of the system is to provide consumers with safe and nutritious food obtained from healthy plants and animals. This policy is based on three main objectives: ensuring that food and animal feed are safe and nutritious, establishing high standards in animal health and plant protection, and providing consumers with transparent information about the origin and content of food. Within this scope, mandatory controls are carried out at all stages of food production and traceability is ensured throughout the food chain.

Food safety cannot therefore be reduced to individual analyses conducted on the final product alone. Risk needs to be managed at its source throughout the chain extending from agricultural production to transport, storage, sale, and household preparation. Good Agricultural Practices, Good Manufacturing Practices, Good Hygiene Practices, and the Hazard Analysis and Critical Control Points System (HACCP) are complementary tools for preventing risks at different stages (Erdoğan, 2026b).

Within this approach, the transport and storage link is as decisive as production. A product produced safely in the field or in an enterprise may become risky at later stages because of a broken cold chain, unsuitable storage, or cross-contamination. Therefore, safeguarding food safety should be addressed through production standards, hygiene, transport conditions, storage capacity, and the continuity of information reaching the consumer. Prevention requires a broader area of responsibility than individual controls conducted at the end of the chain (Erdoğan, 2026b).

7.3. Plant Health, Resource Efficiency, and Continuity of Supply

Maintaining continuity in production depends on the timely identification of plant health risks. The spread of pests and diseases can affect not only the yield of a specific product, but also producers' costs, product quality, export opportunities, and supply planning. Therefore, early warning, accurate diagnosis, integrated pest management, and resistance management

are the practical tools linking food safety and food security. The assessment by Erdoğan and Karasungur (2025) of next-generation agricultural practices shows that digital monitoring and decision-support tools contribute to the capacity to identify risks early.

From the perspective of plant health, it cannot be expected that residue risk will be completely eliminated at the consumer stage; prevention begins during production. Therefore, accurate diagnosis, the selection of the appropriate plant protection product, application at the correct time and dose, and the use of suitable equipment should be assessed together in terms of both continuity of production and food safety. Strengthening integrated pest management and biological and biotechnological methods are complementary tools that reduce dependence on chemical control (Erdoğan, 2025).

The same relationship applies to resource efficiency. Water, fertilisers, and plant protection products are necessary inputs in production. However, when used at the wrong time, at the wrong dose, or outside the intended target, they can increase both costs and environmental pressure. Precision practices do not aim for producers to abandon these inputs altogether, but to use them in a manner compatible with need and capable of verification. The value of this approach in terms of resilience lies in its potential to reduce input costs while addressing pressure on soil, water, and biodiversity.

For this reason, the European Green Deal and the Farm-to-Fork approach are not merely matters of export regulation for Türkiye. Erdoğan et al. (2024) emphasise that climate neutrality, the circular economy, biodiversity, and sustainable agriculture objectives are related to Türkiye's agricultural competitiveness. The resilience perspective makes it possible to regard these external regulations not as a burden of compliance, but as an opportunity to make the production system more efficient, traceable, and better prepared for risks.

7.4. Local Capacity and Regional Differentiation

Although food-system decisions are taken at the national level, their outcomes often emerge at the regional level. Water constraints, production patterns, transport opportunities, storage infrastructure, market access, and producer organisation vary among provinces. Therefore, every policy developed for resilience requires a monitoring framework that makes regional

capacity visible. The fact that the same support instrument does not produce the same result for the same product in different regions is a natural consequence of these local conditions.

Strengthening local capacity does not mean merely increasing production. Elements such as cooperatives, shared machinery use, local storage, cold chains, advisory services, and women's and young producers' access to finance are also part of capacity. The emphasis on social and institutional dimensions in the report of the Ministry of Agriculture and Forestry (2021) clearly shows that resilience cannot be limited to technical solutions.

Within this framework, cooperative-based shared technology use and leasing models offer feasible options, particularly for small producers. The purchase of drone, sensor, or data-analysis infrastructure by a single enterprise may not always be economically viable; however, making these tools accessible through shared use can reduce technology costs. For such models to be effective, technical support, maintenance and repair, and practical knowledge must be organised alongside the provision of equipment (Demir, 2026).

8. FOOD LITERACY AND THE CONSUMER PERSPECTIVE

8.1. The Scope of Food Literacy

Food literacy should not be defined merely as having knowledge about nutrition. The Türkiye Food Literacy Strategy and Action Plan defines this concept as the ability to access, understand, and evaluate information on food and nutrition; transform it into consumption habits by making informed decisions; and select and consume an appropriate amount of safe and healthy food (FAO, 2023). This framework encompasses label reading, access to reliable information, food preparation skills, the reduction of food loss and waste, and sustainable consumption behaviours.

Under inflationary conditions, consumer decisions are determined more by price. However, focusing solely on the lowest price may lead to choices that are more costly or of lower quality in terms of product content, nutritional value, portion size, storage conditions, and period of use. The ability to evaluate label information, understand the difference between the use-by date and the

best-before date, purchase only what is needed, and develop appropriate storage practices is therefore important both for household budgets and for preventing waste.

Food literacy does not resolve the problem of economic access on its own. Macroeconomic and sectoral policies are necessary to address exchange-rate pass-through, production costs, and supply-chain disruptions. Nevertheless, literacy supports consumers in becoming more resilient to misinformation, distinguishing reliable sources, and making more balanced decisions with limited resources. FAO (2023) identifies the prevention of misinformation and misleading information, the improvement of label-reading knowledge, and practices aimed at different target groups, including vulnerable groups, among the priorities of the action plan.

Food literacy is not a concept limited to hearing accurate information. Its everyday expressions include the ability to plan before shopping, evaluate the content and storage conditions stated on the label, consider appropriate portions and amounts of use, understand the difference between the use-by date and the best-before date, and select reliable sources of information. Aktaş and Özdoğan (2016) define food and nutrition literacy not only in terms of accessing and evaluating information, but also through the capacity to make healthy choices, understand how the food system functions, and translate this knowledge into behaviour.

One of the most concrete examples of these skills is correctly understanding the difference between the use-by date and the best-before date. Research shows that 72 percent of consumers in Türkiye do not know the difference between these two dates (Food Safety Association [GGD], 2019). The use-by date applies particularly to perishable foods such as meat, milk, and fish. Once this date has passed, the product is not considered safe and should not be consumed, even if its appearance or smell seems normal. The best-before date, on the other hand, refers more to the period during which products such as dry foods, pulses, pasta, and biscuits retain their best quality characteristics. After this date has passed, the product may not necessarily be spoiled; however, changes in taste, smell, and nutritional value may occur.

Misinterpreting date information can lead to products that can be safely consumed being unnecessarily discarded. Yet, what is wasted is not only the food itself; the water, soil, energy, fertiliser, and labour used in producing that

product are also expended without return. Therefore, label reading is a skill that both protects food safety and reduces food loss and waste at the household level.

Food-related risks also arise during food preparation and storage at home. Preparing raw and cooked foods on the same surfaces, leaving products at unsuitable temperatures, and failing to maintain the cold chain are common mistakes. The fundamental approach in food safety is prevention, and the actions that can be taken after a product has spoiled are limited. Proper storage, proper preparation, and label-reading habits are therefore fundamental elements that complement controls and practices at the inspection and production stages.

This approach also links food literacy with the prevention of waste and sustainable consumption. Purchasing only what is needed, storing products correctly, safely using leftover food, and accurately interpreting the information on packaging are behaviours that may appear minor from the perspective of household budgets but are effective in aggregate. However, these skills do not replace access to affordable and nutritious food. Literacy is a complementary capacity that supports more informed decision-making with limited resources, rather than substituting for economic access.

8.2. Institutional Responsibility and the Translation of Knowledge into Behaviour

The definition of Aktaş and Özdoğan (2016) emphasises that literacy is a combination of knowledge, skills, attitudes, and behaviours. This approach shows that public communication cannot be considered merely an activity of "providing accurate information." The message needs to be understandable, relate to the target group's everyday decisions, be delivered through reliable channels, and offer applicable alternatives. Otherwise, information that is scientifically accurate may not find a practical response in consumers' lives.

8.3. The Limits and Potential of Food Literacy Interventions

The most common mistake in designing food literacy programmes is to equate knowledge directly with behavioural change. However, an individual's ability to read a label does not mean that they can make a healthy choice under all circumstances. Income, time, cooking facilities, transport, household

responsibilities, and the quality of food options in the living environment shape behaviour. The systematic scoping review protocol of Manna, Vidgen, and Gallegos (2024), which aims to examine food literacy interventions among adults, proposes that interventions be addressed in the areas of planning and management, selection, preparation, and consumption.

These four areas also provide a functional framework for Türkiye. Planning includes preparing a needs list and managing the budget; selection involves evaluating product content and safety alongside price; preparation includes storage and hygiene knowledge and consumption encompasses decisions about portions, waste, and dietary diversity. Thus, food literacy moves beyond an abstract discourse of awareness and becomes a set of observable and supportable behaviours in everyday life.

The systematic review by Lee et al. (2026) shows that, in most studies, there is an inverse relationship between food literacy and food insecurity, although this relationship is affected by sociodemographic, economic, and cultural conditions. Therefore, programmes should not be designed around a single consumer profile. Students, older people, households with children, individuals living alone, producer households in rural areas, and low-income urban households have different information needs, time constraints, and access opportunities. Label-reading skills are valuable for a household living under price pressure. However, the effect of this skill is not independent of food accessibility, time constraints, kitchen equipment, and income level.

8.4. Misinformation, Labels, and the Relationship of Trust

Misinformation in the field of food can affect consumer decisions more strongly, especially during periods of increased price pressure. Consumers may encounter content that promises quick solutions, creates fear, or presents products as different from what they actually are. The Türkiye Food Literacy Strategy and Action Plan attaches importance not only to the ability to access, understand, and evaluate information, but also to reducing misleading information (FAO, 2023). Within this framework, label reading is not only the ability to understand an ingredient list, but also the ability to distinguish reliable sources of information.

For label information to be effective, the data on products need to be clear, understandable, and comparable. Expecting consumers to decipher

complex information means transferring the responsibility for communication entirely to households. Public authorities, producers, retailers, and civil society share responsibility for providing simple information, reliable guidance, and monitoring misleading claims. Food literacy does not eliminate this shared responsibility; it strengthens consumers' capacity to demand and monitor within the system.

The Türkiye Food Literacy Strategy and Action Plan highlights the reduction of misinformation, the development of label-reading skills, education for different target groups, and the prevention of food loss and waste (FAO, 2023). This approach shows that literacy is not an educational activity directed only at school-age individuals. Designing activities in schools, universities, health institutions, and retail outlets to create shared learning spaces rather than disconnected campaigns can produce more lasting results.

The success of such programmes should not be evaluated solely by the number of participants. Indicators such as shopping planning, the use of label information, storage behaviour, the level of waste, the ability to distinguish reliable sources, and the preservation of dietary diversity also need to be monitored. In this way, food literacy can be assessed not merely as knowledge transfer, but as a behavioural capacity that strengthens consumers' position within the food system and operates alongside public policies.

8.5. Implementation Conditions and Adaptation to Target Groups

Consumers' decisions about food often appear to be instant choices made at the store shelf. However, these choices are shaped not only by income but also by the time available for preparing food at home, kitchen equipment, transport, childcare, working hours, and the quality of retail outlets in the living environment. Education delivered without taking these conditions into account may fail to translate into behaviour even when it conveys accurate information. For this reason, efforts to strengthen food literacy need to consider households' actual daily rhythms and constraints on access. The systematic scoping review protocol of Manna et al. (2024) also aims to evaluate food literacy interventions across different socioeconomic groups.

For example, planned shopping is not merely a matter of budget discipline. The presence of affordable and reliable retail outlets nearby,

conditions for storing products, and the availability of time for food preparation at home determine whether this behaviour is feasible. Similarly, the seasonal and regional availability of fresh products affects households' capacity to maintain dietary diversity. These relationships make visible the link between food literacy and urban planning, retail structures, and social policy.

For this reason, programmes need to be differentiated according to target groups. Practical preparation and budget planning may come to the fore for students; storage and access to reliable information for older individuals; menu planning and waste prevention for households with children; and different content related to both production and consumption decisions for producer households. Rather than disseminating uniform messages, an educational design that adapts shared basic principles to different living conditions produces more realistic results.

FAO's (2023) action plan offers a framework for accessing food and nutrition information, evaluating that information, and developing practices for different target groups. Instead of using a prescriptive language that tells individuals which product they should choose, education should build their capacity to evaluate information, distinguish risks, and make more balanced decisions within their own circumstances. In this way, food literacy moves beyond being the subject of temporary campaigns and becomes an area of skill that develops throughout life.

8.6. Protecting Dietary Quality under Price Pressure

During periods of high food prices, households often have to establish a difficult balance between quantity and quality. Choosing cheaper products may ease budget pressure in the short term, but it can have long-term consequences if it pushes dietary diversity, product quality, or safe storage conditions into the background. FAO, IFAD, UNICEF, WFP, and WHO (2025) demonstrate that high food prices are closely associated with economic access to a healthy diet. Therefore, dietary quality is not a secondary outcome of discussions on inflation, but a central indicator.

At this point, it is not sufficient simply to call on consumers to save money. Access to budget-friendly and nutritious options, knowledge of seasonal products, proper storage, portion management, and the reduction of waste are important. However, for these measures to be effective, the market

needs to offer safe and accessible options. Efforts to address food prices should not rest the burden on households entirely through individual skills. Social support, public procurement, school nutrition, and local access mechanisms should also be considered as part of this framework.

The role of food literacy at this point is to prevent consumers from remaining vulnerable in the face of misinformation. Price-focused misleading claims, promises of miracle products, or fear-based discourses without scientific basis can find a more receptive audience, especially during periods of economic uncertainty. A consumer who is more capable of evaluating labels, official information, and reliable expertise has a greater opportunity to direct limited resources towards healthier and safer choices (Aktaş & Özdoğan, 2016; FAO, 2023).

8.7. Trust and Governance in the Food System

Resilient food systems are not established solely through well-designed technical tools; the governance capacity that ensures the continuity of these tools is also decisive. Clear division of responsibilities among institutions, regular data sharing, predictability of support mechanisms, and the participation of producer and consumer organisations in decision-making processes increase the reliability of policy implementation. The approach of Zurek et al. (2022) draws attention to the fact that resilience requires different actors to adapt their activities towards shared objectives.

Policy continuity is particularly critical in agricultural production. Producers make decisions regarding production patterns, irrigation investments, orchard establishment, machinery purchases, or the use of digital technology based not on short-term signals but on medium- and long-term expectations. Frequently changing support schemes, uncertain market information, or disruptions in advisory services reduce the capacity to take risks. In contrast, predictable support and reliable information make it easier for producers to invest in climate adaptation and resource efficiency.

The consumer dimension of food-system governance is equally important. The transparent provision of information on price, quality, safety, and labels strengthens consumer trust and market accountability. Transparency does not reduce prices on its own, but it reduces information asymmetry and

limits the effect of misleading practices. In this respect, resilience is a matter of both material capacity and relationships of trust.

8.8. The Need for Research and Monitoring

A single index is not sufficient to monitor the resilience of the food system. Indicators related to production, water use, input costs, product loss, logistics performance, price transmission, access to nutritious food, and consumer behaviour need to be assessed together. Each indicator answers a different question, however, when the links among them are not established, policymakers may intervene only in the visible side of the problem. As Zurek et al. (2022) point out, measuring resilience without clarifying the outcome for which it is intended to be strengthened may reduce the concept to a superficial label.

Bringing food inflation and food-system resilience together within the same monitoring framework in Türkiye is particularly useful in terms of policy coherence. TÜİK price data, agricultural production and input data, climate and water indicators, plant health monitoring results, food loss and waste records, and household nutrition indicators can complement one another. Such a framework enables the earlier identification of whether an improvement in one area creates an unforeseen cost in another. For example, an increase in production alone is not sufficient in terms of resilience if it intensifies pressure on water or fails to resolve the problem of access.

Another dimension of the need for research is monitoring the effects of food literacy programmes. Although changes in knowledge levels after education are important, it should also be assessed whether a programme creates a lasting difference in shopping planning, storage, waste, label use, and dietary diversity. The findings of Lee et al. (2026) show that comparable measurements and longitudinal studies are needed to better explain the relationship between food literacy and food insecurity. Thus, food literacy moves beyond being a well-intentioned awareness activity and becomes a measurable component of evidence-based public policy.

9. CONCLUSION AND ASSESSMENT

Interpreting food inflation solely as a price increase on market shelves renders invisible the causes of the problem that extend from production to consumption. While post-pandemic supply problems, geopolitical

uncertainties, energy and fertiliser costs, and climate change constitute global pressures, exchange-rate pass-through, dependence on imported inputs, price inertia, supply-chain inefficiency, and income disparities amplify this pressure in Türkiye. Therefore, price stability is an inseparable part of building resilient food systems (Erdoğan, 2026a).

The resilience of the food system requires the joint management of the relationship among reliable production capacity, the protection of natural resources, continuity of supply, and society's access to nutritious food. Strengthening local production capacity, investing in productivity, reducing food loss and waste, adapting to climate change, establishing transparent and traceable supply chains, and ensuring small producers' access to technology are complementary elements of this framework (Demir, 2026; Ministry of Agriculture and Forestry, 2021).

There is a need for a policy mix that protects access to safe and nutritious food in the short term, strengthens producer, supply-chain, and logistics capacity in the medium term, and takes water, soil, biodiversity, and social equity into account in the long term. Recommendations concerning collective purchasing and supply consortia, multiple transport routes, cold chains, domestic fertiliser production, seed development, and drought-resistant varieties show that reducing cost pressure and ensuring continuity of supply need to be considered together (Serttaş & Türk, 2025).

Food literacy is not a policy area that replaces structural problems related to prices and access. It is an empowerment tool that complements them. The capacity of consumers who can evaluate information, read labels, store food properly, and reduce waste to make stronger decisions should be supported by fair prices, safe food, accessible sales channels, and an open information environment.

Within this framework, the priority is to strengthen production and monitoring capacity that identifies water, climate, plant health, and input risks early; to implement traceability, hygiene, storage, transport, and risk-based inspection practices together throughout the chain from production to consumption; and to complement social and economic tools that protect access to safe and nutritious food under price pressure with food literacy initiatives. Addressing these three areas together brings the food safety and food security together within the same resilience framework.

In practice, the first step is to link every intervention to a framework that clearly identifies which risk it will reduce and which outcome it will protect. When resilience is measured only by production volume, consumers' access to nutritious food, the continuity of small producers' incomes, or impacts on natural resources may remain invisible. Therefore, indicators related to water, input costs, product loss, logistics, price transmission, and economic access need to be assessed within the same monitoring system. As Zurek et al. (2022) emphasise, the outcome to be protected, the shock to be faced, the group to be affected, and the time horizon should be defined at the outset of a resilience assessment.

In the short term, it is important to consider multiple transport routes and long-term capacity agreements against freight and supply disruptions, as well as to examine the feasibility of collective purchasing and supply consortia and option-based price contracts for input costs. These tools can help limit the reflection on producers and consumers of sudden fluctuations, particularly in imported inputs and trade corridors. During the same period, developing alternative markets for products with high market concentration offers an area of implementation that can reduce risk on both the supply and sales sides (Serttaş & Türk, 2025).

In the medium term, strengthening cold-chain, storage, and processing capacity should be regarded as a priority for reducing product loss, preserving quality, and limiting the effects of price fluctuations. Designing these investments together with certification, technical advice, and small producers' access to markets ensures that the focus is not limited to creating infrastructure alone. Developing domestic fertiliser production capacity should also be considered a structural step to reduce cost pressure arising from external dependence (Serttaş & Türk, 2025).

The 2025 report of FAO and partner organisations recommends that intervention against food inflation should not be limited to temporary price-suppression tools, but should address targeted social protection, coordination of fiscal and monetary policies, market transparency, strategic reserves, and investment in resilient agri-food systems together (FAO et al., 2025).

In the long term, domestic seed development, biotechnology, drought-resistant varieties, water efficiency, and early-warning infrastructure should be considered not as separate areas, but as components of climate-adapted

production capacity. This approach is also consistent with the international food-system framework, which addresses the objective of maintaining continuity of production together with the protection of natural resources and economic access to a healthy diet (FAO, IFAD, UNICEF, WFP, and WHO, 2025).

On the consumer side, the practical implication is to support individuals who can correctly interpret label and date information, store food under safe conditions, and distinguish misinformation. However, these efforts do not replace social and market policies that ensure economic access to safe and nutritious food. Food literacy should be considered a complementary capacity that strengthens the effects of these policies at the household level (FAO, 2023).

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CHAPTER 12

INSECTICIDE RESISTANCE AS A SILENT THREAT: RESISTANCE MANAGEMENT BASED ON MODES OF ACTION[1]

Pesticide Resistance: Is It a Manageable Risk?

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1. INTRODUCTION

Pesticides are chemical or biological substances used to control weeds, insect pests, and disease agents such as bacteria and fungi that cause yield and quality losses in crop production. However, their improper, uninformed, or excessive use can have a range of adverse effects on human health, the environment, and ecosystem health (Delen et al., 2005; Erdoğan, 2024a, 2024b; Erdoğan et al., 2024; Erdoğan & Bilgin, 2025; Tiryaki et al., 2010). Pesticides should therefore be used within the framework of Integrated Pest Management, in a knowledge-based and environmentally compatible manner that protects biodiversity (Erdoğan, 2021, 2024a, 2024b, 2025a, 2025b; Erdoğan & Bilgin, 2025). The earliest known records of pesticide use appear in Sumerian tablets dating back approximately 4,500 years. These tablets indicate that sulfur was used to control insects. It is also known that the Chinese used arsenic and mercury compounds against insects about 3,100 years ago (Erdoğan, 2025a).

The twentieth century was an important period in the development and widespread use of pesticides (Daş & Aksoy, 2016). Paul Müller was awarded the Nobel Prize for his work contributing to the development of DDT, regarded as a forerunner of synthetic pesticides. The introduction of DDT accelerated the industrial-scale production of pesticides.. Today, pesticides are widely used in agricultural production, and this use is expected to continue in the future (Erdoğan, 2024b; Erdoğan & Bilgin, 2025; Özercan & Taşçı, 2022).

Awareness of the effects of pesticides on the environment and human health increased markedly with Rachel Carson's Silent Spring, published in 1962. This growing awareness laid the groundwork for the banning of pesticides in the Persistent Organic Pollutants (POPs) group. In the United States, the agricultural use of DDT was banned in 1972 because of its adverse environmental and health effects. The U.S. Environmental Protection Agency (EPA) ended most domestic uses of DDT on 31 December 1972.

Table 1 presents the dates on which pesticides in the Persistent Organic Pollutants (POPs) group were banned in Türkiye. Accordingly, the use of DDT was restricted in 1978 and completely banned in 1985.

Table 1. Persistent Organic Pollutants (POPs) pesticides banned in Türkiye

Active ingredient	Date banned
Aldrin	1979
Chlordane	1979
DDT	1985
Dieldrin	1971
Heptachlor	1979
Endrin	1979
Toxaphene	1989

Nevertheless, the repeated use of the same active ingredients or products with the same mode of action leads to reduced susceptibility, that is, the development of resistance, in target pest populations. This process is not limited to reduced application success; it increases production costs, narrows control options, and has wider consequences for human, animal, and environmental health.

2. DEVELOPMENT AND CONSEQUENCES OF RESISTANCE AND ITS IMPORTANCE IN VECTOR-BORNE DISEASES

It is evident that pesticides will continue to be used in agricultural production in the near future. Today, approximately one-third of agricultural production is achieved through pesticide applications. In the absence of pesticides, losses of 78% in fruit production, 54% in vegetable production, and 32% in cereal production are anticipated. The ease of application compared with other control methods, their rapid effects, and their ability to control target pests have contributed to the widespread use of pesticides. Insecticide resistance is defined as the persistent failure to achieve the expected level of control, even when a product is used in accordance with its label recommendations, as a result of heritable changes in a pest population (IRAC, 2024). This phenomenon, known in insects for more than a century, has become

a major threat to modern agriculture, crop production, and public-health practices (Mota-Sanchez, 2020).

Insecticide resistance was first documented by Melander in 1914. The study entitled “Can Insects Become Resistant to Sprays?”, published in the Journal of Economic Entomology by Axel Leonard Melander, an entomologist at the Washington Agricultural Experiment Station, is regarded as the first scientific publication to address the development of insect resistance to insecticides (Melander, 1914). Melander observed that some San Jose scale populations had become less susceptible to a lime-sulfur mixture than they had been previously. Earlier trials conducted in Wawawai, Washington, had reported this mixture to be highly effective against the San Jose scale. In contrast, Melander found that approximately 90% of the specimens treated in Clarkston survived. Even after increasing the amount of active ingredient tenfold, he determined that 74% of the scale insects remained alive (Melander, 1914).

From the first record of resistance until 1946, insecticide resistance was reported in a total of 11 pest species. However, the development of synthetic organic insecticides such as DDT, cyclodienes, and organophosphorus insecticides during the 1940s led to the widespread adoption of chemical control. As the same active ingredients came to be used intensively and repeatedly, the resistance problem spread much more widely. The extent of resistance development, in terms of both species and active ingredients, is clearly apparent from the species presented in Table 2.

Table 2. Arthropod species with the highest numbers of reported resistance cases (Sparks & Nauen, 2015)

Species	Common name	Order	Active ingredient[1]	Case[2]
Tetranychus urticae	Two-spotted spider mite	Acari	96	558
Plutella xylostella	Diamondback moth	Lepidoptera	101	1056
Myzus persicae	Green peach aphid	Hemiptera	87	514
Musca domestica	House fly	Diptera	66	463
Bemisia tabaci	Cotton/tobacco whitefly	Hemiptera	69	770
Leptinotarsa decemlineata	Colorado potato beetle	Coleoptera	56	306
Aphis gossypii	Cotton aphid	Hemiptera	52	431
Panonychus ulmi	European red mite	Acari	48	203
Helicoverpa armigera	Cotton bollworm	Lepidoptera	55	892
Rhipicephalus microplus	Southern cattle tick	Ixodida	50	562
Blattella germanica	German cockroach	Blattodea	45	299
Spodoptera litura	Tobacco cutworm	Lepidoptera	46	720

Note. [1] Number of different active ingredients for which resistance has been reported.
[2] Number of different resistance cases reported for each species in the APRD.
Source: Prepared on the basis of Sparks and Nauen (2015) and APRD notes.

According to data from the Arthropod Pesticide Resistance Database, as of 2024, 18,871 insecticide-resistance cases have been recorded worldwide in approximately 632 arthropod species against 364 compounds (Payumo et al., 2024).

Insecticide resistance is not confined to agricultural pests. The development of resistance in mosquitoes, sand flies, ticks, fleas, and other vectors directly affects programmes designed to control vector-borne diseases.

Vectors can transmit disease agents acquired from infected people or animals to other hosts during subsequent feeding. Some freshwater snails also serve as intermediate hosts in disease cycles.

Table 3. Insects and other animals that may act as disease agents in humans (General Directorate of Health for Borders and Coasts of Türkiye, 2026; Erdoğan, 2025a)

Vector or intermediate host	Major diseases
Freshwater snails	Schistosomiasis
Black flies (Simulium species)	River blindness
Fleas	Plague
Mosquitoes (Aedes)	Yellow fever, dengue fever, Rift Valley fever, chikungunya disease
Mosquitoes (Anopheles)	Malaria, lymphatic filariasis
Mosquitoes (Culex)	Japanese encephalitis, West Nile fever, lymphatic filariasis
Sand flies	Leishmaniasis, sand fly fever (Phlebotomus fever)
Ticks	Crimean-Congo haemorrhagic fever, Lyme disease, spring-summer encephalitis, relapsing fever, spotted fever, and tularemia
Tsetse flies	Sleeping sickness (African trypanosomiasis)
Triatomine bugs	Chagas disease (American trypanosomiasis)

Note. The vector–disease associations presented in the table were prepared on the basis of the General Directorate of Health for Borders and Coasts of Türkiye (2026) and Erdoğan (2025a).

Insecticide resistance in vectors can weaken the effectiveness of control programmes intended to prevent disease transmission. According to the World Health Organization's global assessment, resistance to at least one pyrethroid in at least one malaria vector was confirmed in 56 (77%) of the 72 countries where resistance monitoring was conducted during 2010–2016. During the same period, pyrethroid resistance was detected at 1,375 (64%) of the 2,145 monitored sites (World Health Organization [WHO], 2018).

Vector-borne plant pathogens and their arthropod vectors should not be considered outside this framework. Vectors carrying plant, animal, and human pathogens should be addressed together because of fundamental similarities in their biology and their shared effects on human well-being. It is therefore important to broaden the One Health approach, which prioritises human and animal health, to include vector-borne plant diseases (Mauck & Eigenbrode, 2024).

"Pesticide resistance is a serious and growing problem that extends beyond producers. It is a global 'Silent Threat."

2.1. Status of insecticide resistance in Türkiye

In Türkiye and worldwide, chemical control is the method most frequently used against diseases, pests, and weeds that affect yield and quality in agricultural production. Accordingly, pesticide use has been increasing (Erdoğan, 2021, 2024a, 2024b, 2025a; Erdoğan & Bilgin, 2025; Özercan & Taşçı, 2022). In Türkiye, 55,374 tonnes of pesticides were used in 2022, 57,766 tonnes in 2023, and 53,515 tonnes in 2024. Pesticide use per hectare is 2.26 kg/ha, a figure lower than that in developed countries. Nevertheless, pesticide use is higher in the Mediterranean, Aegean, and Marmara regions, where agricultural production is intensive.

A total of 669 harmful organisms have been identified in Turkish agriculture, and control measures are applied against approximately 350 of them. Pesticides are used not only in agricultural production but also in public health to control vector insects. Pesticides used outside agriculture are defined as biocides and are used in homes to repel or eliminate unwanted or harmful organisms. In many developing countries, they are also widely used against disease-causing organisms or vector species that carry these pathogens.

In Türkiye, plant protection products to be used within Integrated Pest Management are determined through risk assessments. These assessments aim to reduce potential risks to human and environmental health by considering active ingredients' by considering the effects of active ingredients on mammals, fish, non-target organisms, and honey bees, as well as their persistence in soil Products such as biological preparations and insect growth regulators are given priority in integrated pest management. Risk assessments of registered pesticides have covered a total of 734 PPPs: 495 active ingredients, 204

mixtures, 13 biological preparations, 21 biological control agents, and 1 safener. Products found suitable are made available to producers through integrated control technical instructions (Erdoğan, 2024a).

2.2. Regulations on resistance management

Regulations on resistance management are an important component of this approach. In the process initiated by the Regulation on the Classification, Packaging and Labelling of Plant Protection Products, dated 23 March 2010 and published in Official Gazette No. 27530 of OJ, labels of plant protection products began to include information on modes of action. This practice continued in subsequent legislative revisions (Erdoğan, 2021). Information on the modes of action of PPPs recommended for integrated control was also added to the revised Integrated Control Technical Instructions (Erdoğan 2024a). Labels clearly state that repeated applications of products with the same mode of action encourage resistance development; therefore, the recommended total number of applications should not be exceeded and, where repeated treatment is necessary, products with different modes of action should be preferred.

Resistance risk is also considered for active ingredients being registered for the first time. Where an application for registration or a request for a new indication is made against pests such as *Aphis gossypii, Bemisia tabaci, Cydia pomonella, Frankliniella occidentalis, Helicoverpa zea, Leptinotarsa decemlineata, Myzus persicae, Tetranychus urticae, Panonychus ulmi, Thrips tabaci, Trialeurodes vaporariorum, Tribolium castaneum,* and *Tuta absoluta*, as well as designated disease agents, submission of a resistance-study report containing baseline toxicity values and prepared in accordance with the relevant principles is required. This practice ensures that resistance is considered from the licensing stage onward.

2.3. Application infrastructure and equipment performance

In resistance management, both product selection and the technical accuracy of application are important. Three test centres have been established in Manisa, Adana, and Antalya to evaluate the performance and application safety of plant protection equipment and machinery in Türkiye. At these centres, spray performance; pressure and flow-rate values; nozzle distribution;

droplet size; airflow and atomisation quality; and the functioning of tanks, agitators, and connecting components are checked. The proper operation of application equipment is important to ensure that PPPs reach the target area uniformly and at the appropriate dose. This helps reduce control failures and resistance-selection pressure that may result from insufficient doses, uneven distribution, or excessive applications.

2.4. Findings on insecticide resistance in Türkiye

The use of plant protection products (PPPs) in Türkiye is an important indicator that should be assessed together with the intensity of agricultural production and the cropping pattern. According to Erdoğan's (2024b) data, total PPP use in 2023 was 57,766 tonnes; insecticides accounted for 21.34%, herbicides for 26.85%, fungicides for 34%, acaricides for 5.37%, and other PPPs for 12%. At provincial level, Antalya stands out as the province with the highest PPP use, at 4,271.889 tonnes and a share of 7.7%.

Temel and Hatipoğlu (2025) report a total of 69 studies and 2,923 case records on insecticide resistance in Türkiye. In this assessment, a case was considered on the basis of the pest species, the active ingredient used, and the region or population combination. The fact that 73% of the studies concerned agricultural pests and 26% concerned public health indicates that resistance is particularly prominent as an issue in agricultural production. Among agricultural pests, the highest concentration of cases is reported in the Mediterranean Region, while Antalya stands out as the province with the highest reported number of cases (Figure 1).

In terms of species, *Bemisia tabaci, Aphis gossypii, Frankliniella occidentalis, Myzus persicae, and Tetranychus urticae* rank first, while Hemiptera accounts for 41% of cases at the order level. Among insecticide groups, neonicotinoids rank first with 286 cases, followed by organophosphates, pyrethroids, and carbamates. At the active-ingredient level, imidacloprid, acetamiprid, and thiamethoxam are among the most frequently reported substances (Temel & Hatipoğlu, 2025).

The "Pesticide Resistance" webpage established by the Department of Plant Protection at Ege University is an important initiative for compiling records on insecticide resistance in Türkiye. The site provides information on provinces where case numbers are concentrated and on the relationships among

pests and active ingredients; in this respect, it functions as a database for Türkiye.

When Erdoğan's (2024b) data on PPP use and Temel and Hatipoğlu's (2025) resistance assessment are considered together, a clear correspondence is evident between use intensity and the geographical distribution of resistance records. Within the Mediterranean, Aegean, and Marmara regions, where PPP consumption is high, the Mediterranean Region and Antalya in particular stand out both in the amount of use and in the number of resistant species and cases. This shows that insecticide resistance is not limited to a single pest species or active ingredient and should be monitored more closely in areas of intensive agricultural production and high PPP use.

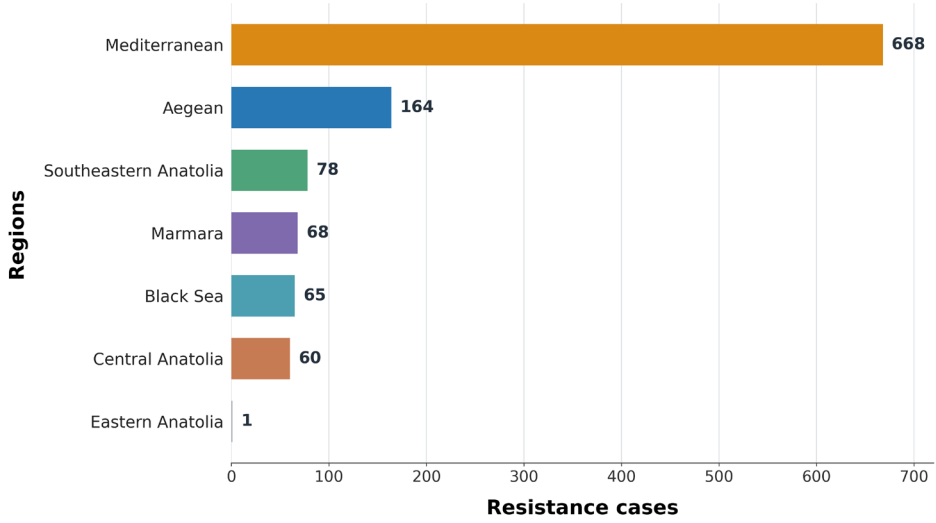

Figure 1. Distribution of resistance cases in agricultural pests by region

Note: Regional case numbers for agricultural pests presented in the presentation were used.

Source: Temel, A., & Hatipoğlu, A. (2025). Studies on insecticide resistance in Türkiye [Presentation].

Resistance management should therefore be carried out through field-based monitoring, assessment at species and population level, correct application techniques, and product selection based on mode of action. The use of insecticides with the same mode of action should be assessed through IRAC groups. It is particularly important to monitor prominent species in the order

Hemiptera together with resistance records for neonicotinoid, organophosphate, pyrethroid, and carbamate groups.

Within this framework, the establishment of a "Scientific Committee for Monitoring Pesticides Resistance" within the Ministry of Agriculture and Forestry is considered necessary to monitor resistance regularly and support management decisions with scientific data.

2.5. Monitoring Application History through B-REÇETE (Plant Prescription)

Türkiye has approximately 24 million hectares of agricultural land, of which 17 million hectares are registered under the Farmer Registration System (ÇKS). Total crop production has reached around 137 million tonnes, while fruit and vegetable production accounts for approximately 55 million tonnes. Within this production system, there are 669 harmful organisms, 348 active substances, and 5,429 registered plant protection products. The country has 8,301 dealers, 17,913 authorised prescription writers, and approximately 495,000 applicators. This scale of agricultural activity makes effective management increasingly critical.

Reliable monitoring of application history is as important to resistance management as selecting the correct active ingredient and mode of action. The B-REÇETE (Plant Prescription) system provides the necessary infrastructure by bringing together, within a single chain of records, the transactions carried out by producers, authorised prescription writers, dealers, and applicators. Recording prescription, sale, application, and harvest information makes it possible to assess retrospectively which product was used on a particular parcel, against which pest, when, and at what dose.

B-REÇETE was introduced as a pilot scheme in the provinces of Mersin, Samsun, Ankara, and Kırklareli on 12 January 2026. In the first phase, electronic prescriptions became mandatory for 349 plant protection products containing five high-risk active substances. The system was rolled out nationwide on 1 July 2026. For the system to operate, producers must be registered in the Farmer Registration System (ÇKS), the Agricultural Production Registration System (TÜKAS), and other relevant registration systems. All plant protection products, whether subject to prescription requirements or not, may be sold only through the system. A prescription must

be issued for active substances subject to prescription control. In particular, prescription use has become mandatory for a total of 286 products containing acetamiprid, abamectin, diflubenzuron, pyriproxyfen, or quinclorac. At the same time, comprehensive training activities are being conducted for farmers, dealers, professional applicators, and local agricultural authorities. B-REÇETE is regarded not only as a mechanism for strengthening food safety in the domestic market, but also as a strategic step towards enhancing Türkiye's credibility in export markets. Reduced uncontrolled pesticide use and stronger traceability are expected to decrease residue-related border rejections and facilitate access to markets with higher standards.

These records are also valuable when resistance is suspected, as they help distinguish the possible causes of application failure. They allow the successive use of active ingredients with the same mode of action under different trade names to be examined systematically, together with application intervals, doses, timing, and target-pest information. In this way, technical application errors can be distinguished more reliably from loss of susceptibility. Where necessary, biological susceptibility tests and other confirmation methods can then be directed to the appropriate areas.

The principal contribution of B-REÇETE to resistance management is that the data can be used not only for inspection, but also to support preventive decision-making. In high-risk crop–pest systems, monitoring repeated use of products from the same IRAC group through application histories can support the development of rotation plans suited to field conditions. Within this framework, the electronic record system should be viewed as a tool that goes beyond sales control in the prescription-writing process and makes it part of a traceable, more rational control programme based on mode of action. Its effective use for this purpose depends on complete and timely data entry, as well as the joint assessment by authorised prescription writers of the pest, crop, application timing, and mode of action. Where a regular flow of information is ensured among producers, dealers, applicators, and advisers, B-REÇETE can contribute not only to reducing residue risk, but also to preventing unnecessary applications and limiting selection pressure on insecticides.

2.6. The cycle of resistance development

Resistance develops when less-susceptible individuals, initially present at low frequency in a natural pest population, gain an advantage after insecticide applications. While the application suppresses susceptible individuals, those carrying resistance-associated traits survive and contribute more to subsequent generations. Repetition of the same selection pressure gradually increases the proportion of resistant individuals in the population. Resistance is therefore the outcome of selection occurring throughout an application history rather than the failure of a single application.

Figure 2 shows the change in population structure resulting from successive applications of an insecticide with the same mode of action. At the outset, susceptible individuals predominate and the level of control achieved by the first application is high. However, continued use of insecticides with the same mode of action gives a selective advantage to resistant individuals that survive. As these individuals transmit resistance genes to subsequent generations, the proportion of susceptible individuals in the population gradually declines; as a result, efficacy, which was 94.75% in the first treatment, may fall to as low as 12.5% in the last treatment.

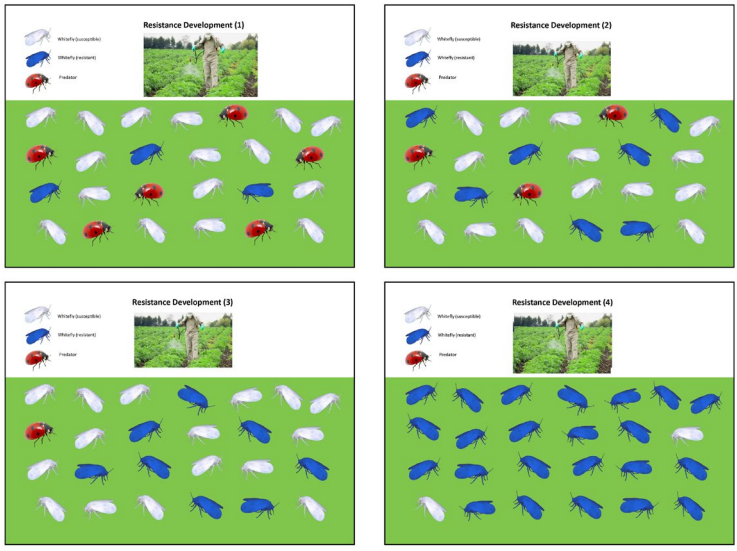

Figure 2. Stages in the development of insecticide resistance (Erdoğan et al., 2024).

Another important point is that, during the same process, the loss of beneficial insects weakens natural pressure on the pest and makes control even more difficult.

The rate at which resistance develops is determined jointly by the number of pest generations, migratory ability, host range, persistence of the insecticide, application dose, and application frequency. Although failure to achieve the expected efficacy under field conditions may raise suspicion of resistance, factors such as misdiagnosis, inadequate coverage, calibration problems, inappropriate application timing, weather conditions, and reinfestation can lead to similar results. A decision that resistance is present should therefore not be based solely on field observation (IRAC, 2024). As resistance develops, the number of applications or the amount of active ingredient used may increase in order to achieve the same level of control. This raises production costs, increases pressure on non-target organisms and biodiversity, and increases the risk of residues and environmental effects. The loss of effectiveness of chemical-control options places both integrated pest-management programmes in agricultural production and interventions aimed at controlling vector-borne diseases at risk.

2.7. The relationship between insecticide resistance and One Health

Insecticide resistance is not merely a technical control problem encountered in agricultural production; it is one of the central issues of the One Health approach, affecting human, animal, plant, and environmental health within the same system. One Health emphasises that these fields cannot be assessed independently and that cross-sectoral cooperation and sustainable practices are needed to protect health. In this context, safeguarding plant health is important for maintaining safe and adequate food production, while the loss of efficacy of insecticides used against pests has multidimensional consequences extending from production to the environment and public health.

The first One Health consequence of this vicious cycle is increased pressure on food safety and human health. Applications made too frequently or at excessive doses may increase residue risk in agricultural products and pesticide exposure among applicators and agricultural workers. In addition, exceeding maximum residue limits may lead to export notifications and

economic losses, affecting both producer income and the sustainability of the agricultural system. Insecticide resistance should therefore be addressed not merely as a problem that increases control costs, but as a public-health issue affecting access to safe food and the continuity of agricultural production (Erdoğan, 2024b; Erdoğan et al., 2024).

Increased insecticide use due to resistance also exerts considerable pressure on environmental health and biodiversity. Plant protection products may be transported into soil and water systems through drift, runoff, irrigation, and precipitation; they may adversely affect soil organisms, natural enemies, parasitoids, predators, and pollinators. Suppression of natural enemies may in turn allow pest populations to increase again, further heightening reliance on chemical control. Resistance, pesticide use, and ecosystem degradation thus become a mutually reinforcing cycle. Breaking this cycle depends not only on suppressing the target pest but also on protecting the integrity of the agroecosystem (Erdoğan et al., 2024).

Insecticide resistance is also of particular importance for public health in vector control. Insecticides remain important tools in interventions against the vectors of malaria, dengue fever, Zika, West Nile virus, cutaneous leishmaniasis, and similar diseases. The development of resistance in vector populations can reduce the efficacy of applications and make disease control more difficult. Insecticides used in agricultural areas and in public-health practices should therefore be considered together in terms of resistance management.

Like antimicrobial resistance, pesticide resistance is a serious and expanding concern. Its consequences extend beyond producers, affecting food production, public health, environmental protection, and society as a whole. For this reason, it should be regarded as a global "silent threat."

Effective resistance management depends on applying the 5R principle: using the right treatment against the correctly diagnosed pest; selecting the right pesticide according to its mode of action; applying it at the right time, at the right dose, and with the right equipment.

This approach prevents unnecessary and incorrect applications, improves application efficacy, and allows pesticide use to be better controlled. In addition, regular monitoring, planned rotation of products with different modes of action, compliance with label recommendations, the use of biological and

biotechnical control methods, and the conservation of natural enemies are required.

3. RESISTANCE MECHANISMS

Insecticide resistance cannot be explained by a single biological process. More than one resistance mechanism be involved in the same species, or even in the same population. Resistance management must therefore determine not only the active ingredient to which a population is resistant, but also the mechanism through which resistance has developed. Resistance mechanisms in insects are classified into four main group (Ünal & Gürkan, 2001).

3.1. Metabolic resistance

In metabolic resistance, the amount or activity of detoxification enzymes such as esterases, glutathione S-transferases, and cytochrome P450 monooxygenases increases. As a result, an insecticide may be metabolised more rapidly or rendered ineffective before it reaches its target site. Enzyme-activity tests and synergist applications are used to assess this mechanism. Biochemical findings, however, must be interpreted together with phenotypic susceptibility data.

3.2. Target-site resistance

In target-site resistance, changes in the protein structure to which the insecticide binds reduce the effect of the active ingredient. Voltage-gated sodium channels, acetylcholinesterase, nicotinic acetylcholine receptors, GABA receptors, and ryanodine receptors are prominent targets in this respect. A change in the target protein may make insecticide binding more difficult or prevent the expected biological response after binding (Junqueira et al., 2024).

3.3. Penetration resistance

In penetration resistance, changes in cuticle structure slow the entry of the insecticide into the insect body. The active ingredient reaches the target site later or in a smaller amount. This mechanism is often observed together with other mechanisms rather than in isolation, such as increased detoxification-enzyme activity. Reaching an effective insecticide concentration then becomes even more difficult.

3.4. Behavioural resistance

In behavioural resistance, the insect reduces its exposure by avoiding the insecticide-treated surface or application area. Movement towards protected areas after treatment, shortening the duration of contact with the surface, or altering feeding behaviour are examples of this mechanism. Detecting this mechanism under field conditions can be difficult because application technique, plant cover, and pest mobility also affect the observed behaviour.

3.5. Cross-resistance and multiple resistance

Cross-resistance occurs when a single resistance mechanism confers protection against two or more insecticides. It is more common among active ingredients that act on the same target site and belong to the same mode-of-action group. For example, when resistance to an organophosphorus insecticide develops as a result of reduced sensitivity of acetylcholinesterase, resistance to another organophosphorus insecticide with the same mode of action may also occur rapidly. Cross-resistance may also occur between mode-of-action groups where the same detoxification enzyme metabolises different insecticides. Increased esterase activity may be considered an example of this situation (IRAC, 2024; WHO, 2016).

Cross-resistance can be can be classified as positive or negative. In positive cross-resistance, a pest population becomes resistant to two or more insecticides through a single mechanism. In negative cross-resistance, a gene that causes resistance to one insecticide may make the population more susceptible to another insecticide.

Multiple resistance is the simultaneous presence of more than one independent resistance mechanism in the same individual or population. Resistance to two insecticides in the same population does not in itself demonstrate multiple resistance; it may also result from cross-resistance. Biological tests, synergist and biochemical tests, and molecular analyses must be evaluated together in order to distinguish between them.

3.6. Mode-of-action classification and resistance management

Insecticide resistance has become a widespread problem, affecting not only producers and consumers but also companies developing plant protection products. For this reason, the Insecticide Resistance Action Committee (IRAC) was established in 1984 by companies in the plant protection products sector.

Once the importance of mode of action in resistance development was understood, IRAC prepared the “Insecticide Mode of Action Classification Scheme” in 1998. The scheme was designed to prevent repeated use of insecticides with the same mode of action and to provide a clear framework for insecticide-resistance-management practices (IRAC, 2024). Biological pesticides were also included in this scheme in 2024.

Within insecticide resistance management, labels of insecticides available on the market state that repeated applications of plant protection products with the same mode of action encourage resistance development. Therefore, the recommended total number of applications within the same season should not be exceeded; if an application must be repeated, products with different modes of action are recommended. This labelling approach helps translate mode-of-action-based rotation into practice.

4. DETECTION OF RESISTANCE

4.1. Field observation and assessment of field efficacy

Failure to achieve the expected level of control under field conditions may be the first indication of resistance. This should not, however, be interpreted as evidence of resistance on its own. Correct identification of the target species, application dose, calibration of spraying equipment, coverage, water quality, weather conditions, application timing, and the possibility of reinfestation should all be reviewed together. This preliminary assessment ensures that subsequent tests are conducted with the correct population and under appropriate application conditions (IRAC, 2024).

4.2. Biological susceptibility tests (bioassays)

Biological tests (bioassays) compare the response of field populations to insecticides at specified doses or concentrations with that of a susceptible reference population or baseline susceptibility values. In dose-response tests, the resistance ratio is calculated from LC_{50} and LD_{50} values. Diagnostic-dose tests, on the other hand, allow more rapid screening of large numbers of populations. In both approaches, the test method should be standardised appropriately for the target species and developmental stage (Blouquy et al., 2021; Halliday & Burnham, 1990).

4.3. Biochemical methods

Biochemical methods are used to assess the amount or activity of enzymes involved in metabolic resistance. Measuring esterase, glutathione S-transferase, acetylcholinesterase, and cytochrome P450 monooxygenase activities provides an important indication of whether an insecticide is being metabolised more rapidly. Experiments with synergists also help assess the contribution of particular enzyme groups to resistance. The relationship between increased enzyme activity and resistance should nevertheless be interpreted together with biological-susceptibility results (Erdogan et. al., 2008, 2021, 2023,2024).

4.4. Molecular methods

Molecular methods are used to identify the presence or frequency of target-site mutations and resistance-associated genes in a population. PCR-based analyses, allele-specific tests, and sequence analysis are powerful tools for monitoring known mutations. Tests targeting only known changes, however, cannot reveal new or as-yet unidentified mechanisms. Molecular findings must therefore be confirmed through phenotypic tests (Erdogan et.al., 2024, Junqueira et al., 2024).

4.5. Integrated evaluation of methods

Using field observation, biological testing, biochemical analysis, and molecular confirmation together makes it possible both to distinguish the cause of control failure and to assess cross-resistance risk more accurately. Susceptibility-test methods published by IRAC strengthen the comparability of results across laboratories and regions (IRAC, 2026).

5. INSECTICIDE MODES OF ACTION AND RESISTANCE MANAGEMENT

Mode of action refers to the biological process in the insect that an insecticide disrupts in order to exert its effect. The IRAC classification groups active ingredients according to their shared target sites and modes of action. Its role in resistance management is to highlight the selection pressure created by successive use of active ingredients from the same group (IRAC, 2024).

Mode of action and chemical group should not be used interchangeably. Active ingredients with different trade names or chemical structures may act on the same target site. In such cases, selection pressure associated with the same mode of action continues even if the product name changes. Conversely, because there may also be a risk of cross-resistance between subgroups within the same main group, an application programme should be evaluated using the IRAC code (IRAC, 2024; Sparks & Nauen, 2015).

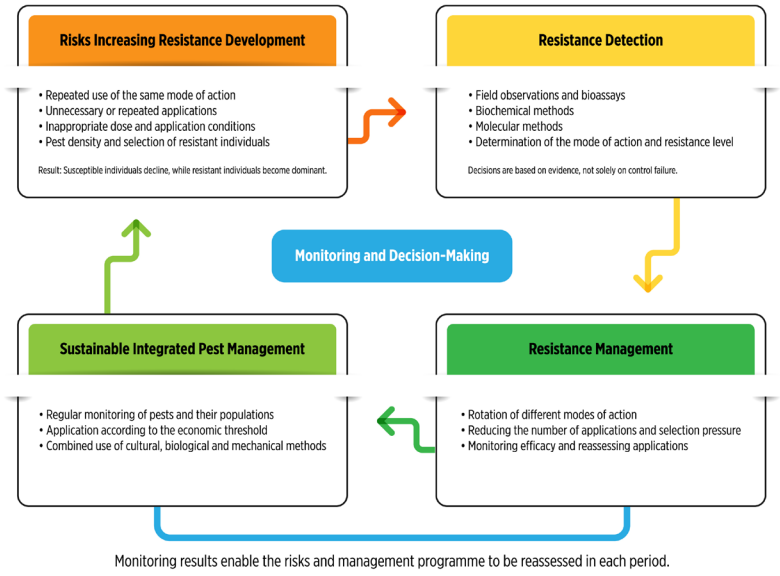

Figure 3. Relationship among risks in insecticide resistance, resistance detection, resistance management, and sustainable integrated control.

Figure 3 illustrates the relationship between the risks contributing to insecticide resistance and the principles of resistance detection, management, and sustainable integrated control. Insecticide resistance is not simply the failure of an active ingredient to provide the expected level of control after application; it is a dynamic process that affects application practices, pest-population structure, and the control programme as a whole. Continuous use of insecticides with the same mode of action, unnecessary applications, and unsuitable conditions of use may accelerate the selection of resistant individuals. Resistance status should therefore be determined by considering field observations together with evidence obtained from biological tests and

biochemical and molecular methods. On the basis of these results, the rotation of insecticides with different modes of action, reduction of application pressure, and regular monitoring of efficacy form the foundation of resistance management. The sustainability of this approach depends on applying integrated-control principles that, rather than viewing chemical control as the sole solution, combine monitoring, economic thresholds, and cultural, biological, and mechanical control methods. Smith et al. (2022) classify modes of action as follows.

5.1. Acetylcholinesterase inhibitors: carbamates (1A) and organophosphates (1B)

Carbamates and organophosphates inhibit the acetylcholinesterase enzyme. When this enzyme is inhibited, acetylcholine accumulates in the synaptic cleft, disrupting nerve transmission and leading to paralysis and death. Because they share the same target enzyme, the risk of target-site-mediated cross-resistance between these groups should be considered.

5.2. Voltage-gated sodium channel modulators: pyrethroids (3A)

Pyrethroids alter the functioning of voltage-gated sodium channels, causing excitation in nerve cells to last longer than normal. Mutations in the sodium channel and metabolic detoxification mechanisms are frequently observed in reduced susceptibility to pyrethroids.

5.3. Nicotinic acetylcholine receptor competitive modulators: Group 4

Neonicotinoids (4A), sulfoximines (4C), and butenolides (4D) disrupt nerve transmission by binding to nicotinic acetylcholine receptors. Because these subgroups act on the same primary target, moving from a 4A active ingredient to one in 4C or 4D does not in every case constitute a true rotation between main groups.

5.4. Nicotinic acetylcholine receptor allosteric modulators: spinosyns (5)

Spinosyns such as spinosad and spinetoram act through a binding site on nicotinic acetylcholine receptors that differs from that of neonicotinoids. By

increasing excitation in the nervous system, they cause cessation of feeding, tremors, and ultimately death.

5.5. Glutamate-gated chloride channel modulators: avermectins (6)

Avermectins such as abamectin act on glutamate-gated chloride channels, increasing chloride influx into nerve and muscle cells. This leads to hyperpolarisation of the cell membrane and suppression of movement and feeding.

5.6. Juvenile hormone receptor modulators: pyriproxyfen (7C)

Pyriproxyfen mimics the action of juvenile hormone and disrupts normal development and metamorphosis in insects. Its effect is particularly evident at the egg, larval, and nymphal stages; application timing must therefore be compatible with the pest's developmental stage.

5.7. Chordotonal organ modulators: Group 9

Group 9 insecticides suppress feeding behaviour by affecting chordotonal organs, which function in the insect's perception of vibration and position. For Group 9 insecticides used to control aphids and whiteflies, successive use of active ingredients within the same main group should be avoided.

5.8. Microbial midgut disruptors: Bacillus thuringiensis (11A)

Products based on Bacillus thuringiensis dissolve in the midgut after oral ingestion by the target larva and damage the gut epithelium. Their efficacy depends on the target species consuming a sufficient amount of the product and being at the appropriate larval stage.

5.9. Chitin-synthesis inhibitors: benzoylureas (15)

Benzoylureas inhibit chitin synthesis. Development cannot be completed because larvae or nymphs are unable to form a new cuticle during moulting. Low direct efficacy in adults is an expected consequence of this group's biological effect and is not, on its own, an indication of resistance.

5.10. Chitin-biosynthesis regulators: buprofezin (16)

Buprofezin disrupts chitin biosynthesis and the moulting process and is particularly effective during the nymphal stages. Assessment of efficacy may be misleading in populations dominated by late stages or adults.

5.11. Moulting disruptors: cyromazine (17)

Cyromazine acts by disrupting moulting and developmental processes in larvae. It is used against selected dipteran larvae, particularly leaf-mining flies.

5.12. Ecdysone receptor agonists: diacylhydrazines (18)

Diacylhydrazines stimulate the ecdysone receptor, causing premature and irregular moulting. They are used especially against lepidopteran larvae because of their larval-stage-specific effects.

5.13. Mitochondrial electron-transport inhibitors: Groups 20, 21, and 25

METI insecticides in Groups 20 and 21 disrupt energy production by inhibiting electron transport in mitochondria. Cyflumetofen (25) is also an option that targets mitochondrial energy metabolism and is used particularly in mite control.

5.14. Voltage-gated sodium channel blocker: indoxacarb (22A)

Indoxacarb blocks voltage-gated sodium channels and interrupts nerve impulse transmission. Because pyrethroids also affect sodium channels, it should be remembered that the effects of these two groups are not the same; nevertheless, they require careful monitoring at the target-site level.

5.15. Lipid-biosynthesis inhibitors: Group 23

Spiromesifen and spirotetramat affect development and reproduction by disrupting lipid biosynthesis. Their effect may vary according to the developmental stage of the target pest.

5.16. Ryanodine receptor modulators: diamides (28)

Diamides such as chlorantraniliprole and cyantraniliprole disrupt calcium balance in muscle cells through ryanodine receptors. Continuous muscle contraction, cessation of feeding, and paralysis result from this effect.

5.17. Chordotonal organ modulators: flonicamid (29)

Flonicamid has a distinct mode of action that affects feeding behaviour through chordotonal organs. The rapid suppression of feeding in piercing-sucking pests is important in reducing crop damage and the risk of virus transmission. Its placement in an IRAC group different from Group 9 requires that this distinction should be taken into account in rotation planning.

Table 4 presents the main insecticide mode-of-action groups and example active ingredients for each mode of action.

Table 4. Main insecticide mode-of-action groups and active ingredients

1,3-dichloropropene	8A	*beta*-Cypermethrin	3A	Chlormephos	1B	Dichlorvos/ DDVP	1B
Abamectin	6	Bifenazate	20D	Chloropicrin	8B	Dicofol	UN
Acephate	1B	Bifenthrin	3A	Chlorpyrifos	1B	Dicrotophos	1B
Acequinocyl	20B	Bioallethrin	3A	Chlorpyrifos-methyl	1B	Dicloromezotiaz	4E
Acetamiprid	4A	Bioallethrin Scyclopentenyl isomer	3A	Chromafenozide	18	Diflovidazin	10A
Acrinathrin	3A			Clofentezine	10A	Diflubenzuron	15
Acynonapyr	33	Bioresmethrin	3A	Clothianidin	4A	Dimethoate	1B
Afidopyropen	9D	Bistrifluron	15	Coumaphos	1B	Dimethylvinphos	1B
Akanthomyces muscarius Ve6	UNF	Borax	8D	Cryolite	8C	Dimpropyridaz	36
		Boric acid	8D	Cyanide	24B	Dinotefuran	4A
Alanycarb	1A	Broflanilide	30	Cyanophos	1B		
Aldicarb	1A	Bromopropylate	UN			Disodium octaborate	8D
Allethrin	3A			Cyantraniliprole	28	Disulfoton	1B
		Buprofezin	16	Cycloprothrin	3A		
alpha-Cypermethrin	3A	*Burkholderia spp.*	UNB			DNOC	13
Aluminium phosphide	24A	Butocarboxim	1A	*Cydia pomonella* GV	31	d-*trans* Allethrin	3A
Amitraz	19	Cadusafos	1B	Cyenopyrafen	25A	Emamectin benzoate	6
Anticarsia gemmatalis MNPV	31	Calcium cyanide	24B	Cyflumetofen	25A	Empenthrin [(EZ)-(1*R*)isomers]	3A
				Cyfluthrin	3A		
Azadirachtin	UN	Calcium phosphide	24A	Cyhalothrin	3A		
Azamethiphos	1B	Carbaryl	1A	Cyhexatin	12B	Endosulfan	2A
Azinphos-ethyl	1B	Carbofuran	1A	Cypermethrin	3A	EPN	1B

Azinphos-methyl	1B	Carbosulfan	1A	Cyphenothrin (1R)*trans*-isomers]	3A	Esfenvalerate	3A
Azocyclotin	12B	Cartap hydrochloride	14			Ethiofencarb	1A
Bacillus thuringiensis	11A	*Chenopodium ambrosioides near ambrosioides* extract	UNE	Cyromazine	17	Ethion	1B
Bacillus sphaericus	11B			d-*cis-trans* Allethrin	3A	Ethiprole	2B
Beauveria bassiana strains	UNF	Chinomethionat	UN	Dazomet	8F	Ethoprophos	1B
Bendiocarb	1A	Chlorantraniliprole	28	DDT	3B	Etofenprox	3A
Benfuracarb	1A	Chlordane	2A	Deltamethrin	3A	Etoxazole	10B
Bensultap	14	Chlorethoxyfos	1B	Demeton-S-methyl	1B	Famphur	1B
Benzoximate	UN	Chlorfenapyr	13	Diafenthiuron	12A	Fatty acid monoesters with glycerol or propanediol	UNE
Benzpyrimoxan	UN	Chlorfenvinphos	1B	Diatomaceous earth	UNM		
beta-Cyfluthrin	3A	Chlorfluazuron	15	Diazinon	1B		
Fenamiphos	1B	Hexaflumuron	15	Methomyl	1A	Phorate	1B
Fenazaquin	21A	Hexythiazox	10A	Methoprene	7A	Phosalone	1B
Fenbutatin oxide	12B	Hydramethylnon	20A	Methoxychlor	3B	Phosmet	1B
Fenitrothion	1B	Hydroprene	7A	Methoxyfenozide	18	Phosphamidon	1B
Fenobucarb	1A	Imicyafos	1B	Methyl bromide	8A	Phosphine	24A
Fenmezoditiaz	4E	Imidacloprid	4A	Metolcarb	1A	Phoxim	1B
Fenoxycarb	7B	Imiprothrin	3A	Methyl isocyanate	8F	Pirimicarb	1A
Fenpropathrin	3A	Indoxacarb	22A	Mevinphos	1B	Pirimiphos- methyl	1B
Fenpyroximate	21A	Isocylcoseram	30	Milbemectin	6	Polydimethylsiloxane (PDMS)	UNM
Fenthion	1B	Isofenphos	1B	Mineral Oil	UNM		
Fenvalerate	3A	Isoprocarb	1A	Monocrotophos	1B	Potassium cyanide	24B
Fipronil	2B	Isopropyl O-(methoxy -aminothio-phosphoryl) salicylate	B	Naled	B	Prallethrin	3A
Flonicamid	9			Neem Oil	NE	Profenofos	1B
Flometoquin	4	Isoxathion	B	Nicotine	B	Propargite	12C
Fluacrypyrim	0C	Kadethrin	A	Nitenpyram	A	Propetamphos	1B
Flubendimide	8	Kinoprene	A	Novaluron	5	Propoxur	1A

Flucycloxuron	5	*lambda*-Cyhalothrin	A	Noviflumuron	5	Prothiofos	1B
Flucythrinate	A	Lepimectin		Omethoate	B	Pyflubumide	25B
Flufenoxuron	5	Ledprona	5	Oxamyl	A	Pymetrozine	9B
Flumethrin	A	Lime sulfur	N	Oxazosulfyl	7	Pyraclofos	1B
Flupyradifurone	D	Lufenuron	5	Oxydemeton-methyl	B	Pyrethrins (pyrethrum)	3A
Fluxametamide	0	Malathion	B	*Paecilomyces fumosoroseus* Apopka strain 97	NF	Pyridaben	21A
Flupyrimin	F	Mancozeb	N			Pyridalyl	UN
gamma-Cyhalothrin	A	Mecarbam	B			Pyridaphenthion	1B
GS-omega/kappa HXTX -Hv1a	2	Metaflumizone	2B	Parathion	B	Pyrifluquinazon	9B
		Metam	F				
Halfenprox	A	Metarhizium brunneun strain F52	NF	Parathion-methyl	B	Pyrimidifen	21A
Halofenozide	8			Permethrin	A	Pyriproxyfen	7C
Heliocoverpa armigera NPV	1	Methamidophos	B	Phenothrin [(1*R*)*trans*-isomer]	A	Quinalphos	1B
		Methidathion	B			Resmethrin	3A
Heptenophos	B	Methiocarb	A	Phenthoate	B		

Rotenone (Derris)	21B	Sulfuryl fluoride	8C	Tetraniliprole	28	Triazamate	1A
Sabadilla extract	UNE	Tartar emetic	8E	*Thaumatotibia leucotreta* GV	31	Triazophos	1B
Silafluofen	3A	*tau*-Fluvalinate	3A			Trichlorfon	1B
Sodium borate	8D	Tebufenozide	18	*theta*-cypermethrin	3A	Triflumuron	15
Sodium cyanide	24B	Tebufenpyrad	21A	Thiacloprid	4A	Triflumezopyrim	4E
Sodium metaborate	8D	Tebupirimfos	1B	Thiamethoxam	4A	Trimethacarb	1A
Spidoxamat	23	Teflubenzuron	15	Thiocyclam	14	Vamidothion	1B
Spinetoram	5	Tefluthrin	3A	Thiodicarb	1A		

Spinosad	5
Spirodiclofen	23
Spiromesifen	23
Spiropidion	23
Spirotetramat	23
Sulfotep	1B

Temephos	1B
Terbufos	1B
Tetrachlorvinphos	1B
Tetradifon	12D
Tetramethrin	3A
Tetramethrin [(1R)isomers]	3A

Thiofanox	1A
Thiometon	1B
Thiosultap-sodium	14
Tolfenpyrad	21A
Tralomethrin	3A
Transfluthrin	3A

Wolbachia pipientis (Zap)	UNB
XMC	1A
Xylylcarb	1A
zeta-Cypermethrin	3A
Zinc phosphide	24A

Source: Prepared on the basis of Smith et al. (2022) and IRAC (2024).

6. APPLICATION WINDOWS, ROTATION, AND MIXTURES

The aim of resistance management is not to eliminate insecticide use altogether, but to use insecticides, when necessary, in a way that reduces selection pressure. Successive generations should not be treated with products having the same mode of action; where treatment is necessary, a different mode-of-action group should be selected. A rotation plan should take account of the crop growth period, the pest's generation time, the persistence of the product applied, and the predominant pest species in the field (IRAC, 2024).

Under the application-window approach, the growing season is divided into intervals based on the time required for the pest to move from one generation to the next or on the effective persistence of the insecticide applied. Although the same mode-of-action group may be used where necessary within a window, the aim is to switch to a different group in the following window. The presence of more than one pest on the same crop makes this planning more complex because each pest has a different biology and exposure period. Tank mixtures or pre-mixes can contribute to resistance management only when each compound is effective against the target pest and there is no adverse cross-resistance risk among the compounds. Individuals resistant to one active

ingredient are expected to be controlled by the other compound in the mixture. Continued use of the same mixture, however, can select resistance to all of its components and should therefore be planned carefully.

6.1. Insect pest resistance management

Insect pests may appear at different times during the crop cycle, and resistance management should therefore be considered when planning a control strategy. When Figures 4, 5, and 6 are considered together, it is recommended that a crop-growing season be divided into "application windows". Figure 4 shows four application windows established according to the crop growth period. Each window should last either for the time required for the pest to complete one generation, from egg or juvenile stage to adult, or for the residual period of a single insecticide application, whichever is longer. Since determining an insect's generation time is not always straightforward, IRAC recommends a 30-day application window for most pests when this information is unavailable, and a 15-day window for aphids and mites.

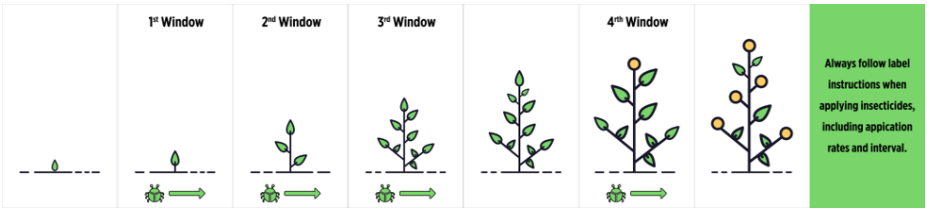

Figure 4. Managing insect pest resistance through application windows*
Source: IRAC (2024)
*Adapted and modified from the *Insecticide Resistance Training Basic Module: Crop Protection*.

The duration of each window should be determined by considering the time required for the pest to complete one generation or the duration of control provided by a single insecticide application. Whichever of these two periods is longer should guide the planning of the application window (IRAC, 2024).

If the pest's generation time is unknown, a 30-day interval may be used for most pests. For aphids and mites, which have shorter development times, 15-day application windows will be more appropriate. Insecticides with the same mode of action, or those associated with cross-resistance, should not be used in adjacent or sequential application windows (Figure 5).

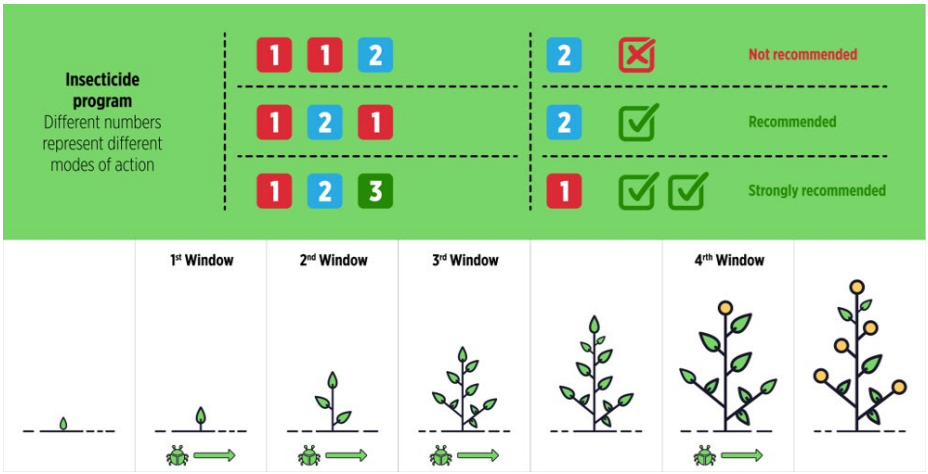

Figure 5. Application-window approach in a production area with a single pest*
Source: IRAC (2024).
*Adapted and modified from the *Insecticide Resistance Training Basic Module: Crop Protection*.

Products from these groups should be considered only in alternate application windows. Where a single pest is targeted in the field, an application programme comprising insecticides with different modes of action should be preferred in order to strengthen resistance management (IRAC, 2024).

As shown in Figure 6, developing a resistance-management strategy for one pest on a crop may be relatively straightforward.

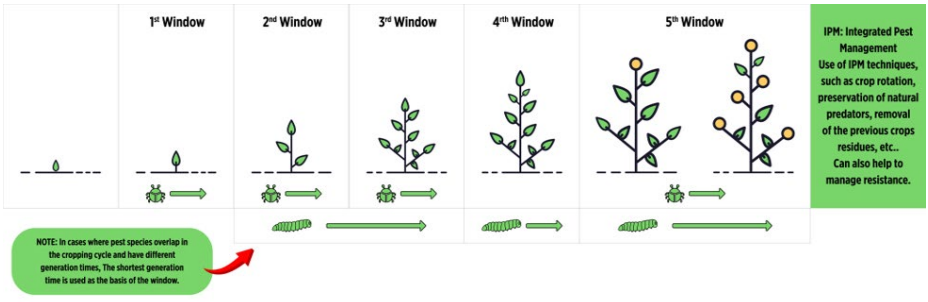

Figure 6. Application-window approach in a production area with multiple pests*
Source: IRAC (2024).
*Adapted and modified from the *Insecticide Resistance Training Basic Module: Crop Protection*.

In practice, however, more than one pest species is often present on the same crop. Application windows must then be arranged to take account of the biology and development time of each pest. This approach can be applied provided that the residual activity of the insecticide used does not exceed the duration of the application window. When the necessary conditions are met, more than one application using products with the same mode of action may also be possible within the same application window (IRAC, 2024).

Once a pest becomes established on a crop or plant, its generations are expected to overlap shortly thereafter. It is therefore not always possible to entirely avoid treating every successive generation with insecticides having the same mode of action. The application-window approach nevertheless provides a practical framework for reducing the likelihood of resistance development. Although pest generation time is affected by temperature and the host plant, fixed application intervals may be used for certain pests in order to develop an application plan.These application scenarios illustrate how rapidly resistance can develop. For example, spinosad was introduced in Hawaii for the control of Plutella xylostella in 1998. The lack of suitable alternatives and the continued weekly use of the product led to resistance developing within approximately two years; consequently, the product lost much of its efficacy and practical value (Zhao et al., 2002).

Similarly, resistance to chlorantraniliprole was observed in P. xylostella populations in China shortly after the product's introduction. This was attributed to the intensive and, in some areas, almost exclusive use of the product for pest control (Wang & Wu, 2012). Other examples of rapid resistance development include resistance to DDT in house flies (Decker & Bruce, 1952), resistance to various insecticides, including pyrethroids, in the Colorado potato beetle (Ioannidis et al., 1991), and pyrethroid resistance in horn fly populations exposed to pyrethroid-treated cattle ear tags and pyrethroid sprays (Sheppard, 1987; Sheppard & Joyce, 1992).

7. RESISTANCE MANAGEMENT WITHIN INTEGRATED PEST MANAGEMENT

Insecticide resistance management is an integral part of integrated pest management. Under the 5R rule, treatment should involve using the right treatment for the correctly diagnosed pest, selecting the right pesticide

according to its mode of action, and applying it at the right time, at the right dose, and with the right equipment. Regular monitoring, decisions based on economic thresholds, correct diagnosis, determination of appropriate application timing, compliance with label doses, cultural measures, the use of resistant varieties, and biological-control practices reduce the need for insecticides and the associated selection pressure. Where chemical control is necessary, the target pest, active ingredient, mode of action, and application history should be evaluated together.

Where appropriate, selecting more selective pesticides that break down more rapidly in the environment contributes to the conservation of natural enemies. Selection should also favour pesticides that have undergone risk assessment and are suitable for use in Integrated Pest Management. Spot, band, or barrier applications can limit the area exposed to insecticides. Equipment calibration, appropriate spray volume, and adequate coverage are as important to application success as active-ingredient selection. Applications below the label-recommended dose may increase resistance risk by allowing more tolerant individuals to survive.

Plant protection products should be applied against correctly diagnosed pests, at the correct time and dose, using calibrated spraying equipment. Appropriate personal protective equipment should be used during application, and the required interval between the last treatment and harvest should be observed. Before a PPP is used, the label approved by the Ministry of Agriculture and Forestry must be read and its recommendations followed carefully. Considering the potential adverse effects of chemical control on human and environmental health, PPPs should be used carefully and within a sustainable, science-based approach that safeguards biodiversity (Erdoğan, 2021, 2024a, 2024b; Erdoğan et al., 2024).

7.1. Preventive practices

The use of pest-resistant varieties, crop rotation, appropriate irrigation and fertilisation, management of alternative hosts in the production area, and, where necessary, soil cultivation can delay the point at which a pest population reaches the economic threshold. Regular field inspections help determine the pest’s developmental stage and whether chemical intervention is genuinely

necessary. Unnecessary applications are thereby reduced, along with selection pressure on insecticides.

7.2. Monitoring, diagnosis, and application quality

Where resistance is suspected, the technical conditions of the application should first be assessed. Field failure should not be interpreted directly as resistance before checking dose, calibration, application volume, coverage, water quality, application timing, and weather conditions. Suspect populations should then be evaluated through standardised biological tests. Monitoring should not be confined to reactive work after application failure has emerged; it should take on a proactive role capable of detecting early changes in susceptibility (IRAC, 2024, 2026).

7.3. Mode-of-action-based rotation

The fundamental rule of rotation is not to apply active ingredients with the same mode of action to successive generations. Even if the product or trade name changes, selection pressure continues when the IRAC mode-of-action code is the same. The decision should therefore be made according to the mode-of-action group, not the active-ingredient name. Application windows should be established by considering the pest's generation time and insecticide persistence, and a different mode of action should be used in the following window (IRAC, 2024; Smith et al., 2022).

7.4. Mixtures and the integrated pest-management approach

In an integrated approach, priority should be given to cultural measures, conservation of biological-control agents, biotechnical methods, selective products, spot or band applications that reduce the area exposed to insecticides, and decisions based on economic thresholds. Pesticides should be applied within the framework of Integrated Pest Management in a knowledge-based, environmentally compatible manner that protects biodiversity (Erdoğan, 2021, 2024a, 2024b, 2025b, 2025c).

Effective resistance management depends on the sound interpretation of field data. Applications undertaken without jointly assessing susceptibility-monitoring results, information on resistance mechanisms, mode-of-action classification, and the biological conditions of the production area may provide short-term control but increase resistance risk in the long term. A well-planned

integrated pest-management programme, by contrast, both manages pest pressure and extends the useful life of insecticides.

7.5. Resistance management within the One Health framework

Management of insecticide resistance should not be regarded solely as an application plan aimed at suppressing the target pest. Increasing application frequency and dose as resistance develops can simultaneously increase residue risk in products, pesticide exposure among applicators, pressure on natural enemies and pollinators, and the likelihood of movement into soil and water ecosystems. Resistance management should therefore be addressed as part of the One Health approach, which safeguards safe food production, environmental sustainability, biodiversity, and public health while protecting plant health.

The first requirement in this framework is that resistance monitoring be conducted to produce regular and comparable data. Field observations and biological susceptibility tests should be evaluated together with biochemical and molecular analyses where necessary. The resulting data should not be treated merely as reactive information to be used in areas where application failure has occurred, but as part of a monitoring infrastructure that provides early warning in high-risk crop–pest systems. This allows the necessary rotations, product selections, and control strategies to be planned before resistance becomes widespread.

Mode-of-action-based rotation is one of the principal tools of this approach. A rotation decision, however, does not simply mean selecting different commercial products. It should be remembered that successive use of active ingredients in the same IRAC group under different names maintains selection pressure. Application windows should therefore be established by jointly considering the application history of the production area, the predominant pest species, the number of pest generations, and product persistence. Mixtures should be used only where each component provides adequate efficacy against the target pest and cross-resistance risk has been assessed carefully.

Another dimension of resistance management from a One Health perspective is the wider adoption of practices that reduce reliance on chemical control. Under the 5R rule, treatments should be applied to using the right

treatment against the right diagnosed pest; selecting the right pesticide according to its mode of action; applying it at the right time, at the right dose, and with the right equipment.Decisions based on economic thresholds, the use of resistant varieties, cultural measures, biological and biotechnical control methods, and conservation of natural enemies reduce insecticide use and, consequently, selection pressure. Where treatment is necessary, the use of the correct dose and timing, suitable equipment, and proper calibration is important both for control success and for preventing unnecessary exposure.

Insecticide applications in agricultural areas should not be considered independently of insecticides used for vector control in public health. In areas where products with the same or similar modes of action are widely used, it is important to monitor resistance in agricultural pests and vector populations together. This approach helps preserve control options in crop production and maintain insecticide efficacy in the control of vector-borne diseases.

Sustained resistance management requires regular information sharing among producers, advisers, applicators, universities, public institutions, and the plant protection sector. The safe collection and disposal of empty plant protection product containers, fertiliser packaging, and other agricultural plastic waste also completes the environmental dimension of this approach (Erdoğan et al., 2024). The success of resistance management depends not only on insecticide selection but also on sound interpretation of field data, application quality, and the shared responsibility of all stakeholders.

It is therefore considered necessary to establish a “Scientific Committee for Monitoring Pesticide Resistance” within the Ministry of Agriculture and Forestry in order to closely monitor and manage resistance.

8. CONCLUSION

Insecticide resistance is not only an application problem but also a heritable and dynamic process that affects the entire production system. Successive use of active ingredients with the same mode of action selects individuals with lower susceptibility and gradually narrows control options. Resistance should therefore be treated not as an outcome recognized only when a product fails to provide the expected effect, but as a risk to be managed through regular monitoring, correct diagnosis, and preventive planning.

An effective programme takes into account the pest's developmental stage, application history, population susceptibility, and the insecticide's mode of action. Combining biological tests with biochemical and molecular methods enables a more accurate understanding of the resistance mechanism. This information makes it possible to plan rotation not merely by changing product names, but across different IRAC groups.

The long-term success of resistance management depends on not treating chemical control in isolation. Regular monitoring, decisions based on economic thresholds, maintenance of application quality, consideration of biological-control agents, and cultural measures reduce selection pressure on insecticides. Monitoring application history through digital record systems such as B-Receipt makes these decisions traceable at parcel and pest level. Such an approach both preserves control capacity in production and helps limit pressure on people, the environment, and biodiversity.

For this reason, managing insecticide resistance is not merely a technical requirement for preserving the efficacy of existing products. It is one of the practical domains of the One Health approach in terms of sustaining safe food production, reducing exposure among applicators and consumers, protecting biodiversity, limiting environmental pollution, and retaining effective tools for vector control.

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CHAPTER 13

HYDROCHAR IN ORNAMENTAL PLANT PRODUCTION: PEAT SUBSTITUTION, GROWING MEDIA PERFORMANCE, PHYTOTOXICITY RISKS, AND SAFE-USE PATHWAYS

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INTRODUCTION

The production of ornamental plants depends heavily on the quality and stability of growing media, because the root-zone environment directly regulates water availability, aeration, nutrient buffering, pH, electrical conductivity, microbial activity, seedling establishment, root development, and final market quality. In containerized ornamental production, the growing medium is not merely a physical support for the plant; rather, it functions as an engineered root-zone system in which water-air balance, soluble salt level, nutrient retention, and biological stability must remain within a suitable range throughout cultivation (Fornes & Belda, 2018; Roehrdanz et al., 2019).

Peat has historically been one of the dominant constituents of horticultural growing media because of its high water-holding capacity, relatively low pH, low nutrient content, and favorable physical structure, which can be adjusted through liming, fertilization, and blending with other materials. However, peat extraction requires bog drainage and mining, which can negatively affect biodiversity and contribute to greenhouse gas emissions, particularly CO_2 and CH_4 (Roehrdanz et al., 2019). Therefore, the development of alternative, renewable, and regionally available growing media has become an important research direction in horticulture and ornamental plant production (Roehrdanz et al., 2019; Farru et al., 2022).

In this context, hydrothermal carbonization products, especially hydrochar, have attracted increasing attention as possible soil amendments and growing medium components. Hydrothermal carbonization (HTC) is a thermochemical process that converts wet organic biomass into a carbon-rich solid product under subcritical water conditions, generally at moderate temperatures and self-generated pressure (Puccini et al., 2018; Wang et al., 2025). Unlike pyrolysis, HTC can process feedstocks with high moisture content without an intensive pre-drying step, which makes it particularly relevant for organic residues such as digestates, municipal green wastes, biosolids, food waste, spent coffee grounds, grape marc, and other wet biomass streams (Farru et al., 2022; Puccini et al., 2018; Wang et al., 2025).

Hydrochar has been proposed as a promising material for circular bioeconomy-based substrate development because it can return carbon and nutrients from organic residues to agricultural or horticultural systems. Depending on feedstock and HTC conditions, hydrochar may contain organic

carbon, macro- and micronutrients, polar functional groups, and hydrophilic surfaces that can influence nutrient exchange, soil organic carbon dynamics, and water-holding behavior (Wang et al., 2025).

Despite this potential, the direct use of fresh hydrochar as a peat substitute or substrate constituent remains challenging (Fornes & Belda, 2018; Puccini et al., 2018; Luutu et al., 2022). Hydrochars may retain labile organic compounds and process-water-derived phytotoxic substances, including organic acids, phenols, furans, furfurals, volatile compounds, and other soluble organics formed during HTC reactions (Farru et al., 2022; Hitzl et al., 2018; Puccini et al., 2018). These compounds can inhibit seed germination, restrict radicle elongation, reduce plant biomass, and create early establishment problems in sensitive species (Puccini et al., 2018; Luutu et al., 2022).

The behavior of hydrochar in growing media is not only a question of chemical toxicity; it is also strongly related to physical and biological root-zone conditions (Fornes & Belda, 2018; Bona et al., 2023). In an ornamental plant study using Calendula officinalis and Petunia hybrida, Fornes and Belda (2018) reported that hydrochar-containing substrates retained excessive water, had poor aeration, and showed high CO_2 concentrations associated with respiratory activity. This finding is relevant for ornamental production, because containerized roots are sensitive to oxygen limitation, waterlogging, excessive microbial respiration, and physical imbalance in the substrate (Fornes & Belda, 2018).

The variability of hydrochar effects has also been confirmed at broader evidence levels (Luutu et al., 2022). In a meta-analysis of 43 published articles and 437 pairwise comparisons, Luutu et al. (2022) found that hydrochar application reduced seed germination by an average of 38% and shoot biomass by 10% across diverse hydrochar properties and experimental conditions. The negative effects were especially associated with high application rates, feedstock type, and the use of unmodified hydrochar, whereas post-treatment significantly mitigated the negative effects on both germination and shoot biomass.

Accordingly, this review evaluates the potential use of hydrochar in agricultural production with a specific focus on ornamental plants, growing media, peat replacement, and safe application strategies. The review synthesizes evidence on HTC production conditions, hydrochar properties,

ornamental and horticultural growing medium performance, peat substitution potential, phytotoxicity mechanisms, nitrogen immobilization, root-zone risks, post-treatment strategies, application rates, and soil amendment functions.

1. Hydrothermal Carbonization and Hydrochar: Concept, Production Conditions, and Material Properties

Hydrothermal carbonization is a thermochemical conversion process in which wet organic biomass is transformed into a carbon-rich solid product under subcritical water conditions (Puccini et al., 2018; Wang et al., 2025). In contrast to pyrolysis, which generally requires relatively dry feedstock and higher processing temperatures, HTC can treat biomass with high moisture content because water acts as both the reaction medium and solvent during conversion (Farru et al., 2022; Hitzl et al., 2018; Wang et al., 2025).

During HTC, organic matter undergoes hydrolysis, dehydration, decarboxylation, aromatization, polymerization, and re-condensation reactions, resulting in three main product fractions: a solid carbonaceous fraction, a process water fraction enriched with soluble organic and inorganic compounds, and a relatively small gas fraction mainly composed of CO_2 (Puccini et al., 2018; Farru et al., 2022). The hydrochar surface may contain oxygen- and nitrogen-containing functional groups, while the process water can contain phenols, furfurals, organic acids, soluble nutrients, and other degradation products formed from carbohydrates, lignin, proteins, and lipids (Farru et al., 2022; Wang et al., 2025).

The characteristics of hydrochar are strongly influenced by HTC process variables, particularly feedstock type, reaction temperature, residence time, and solid-liquid ratio (Wang et al., 2025). Table 1 summarizes the main HTC process variables and their relevance for hydrochar use in ornamental growing media.

Table 1. Major HTC process variables and their relevance for hydrochar use in growing media

HTC variable	Main influence on hydrochar	Relevance for ornamental growing media	Key sources
Feedstock type	Determines ash content, nutrient composition, organic compounds, possible contaminants, and substrate behavior.	Feedstocks such as green waste, digestate, spent coffee grounds, grape marc, biosolids, or municipal residues may produce hydrochars with different phytotoxicity and nutrient profiles.	Farru et al. (2022); Wang et al. (2025)

HTC variable	Main influence on hydrochar	Relevance for ornamental growing media	Key sources
HTC temperature	Strongly affects carbonization degree, functional groups, volatile compounds, nutrient forms, and stability.	Temperature can influence whether hydrochar behaves more as a nutrient source, a carbon amendment, or a phytotoxic material.	Puccini et al. (2018); Wang et al. (2025)
Residence time	May affect hydrochar maturation and chemical transformation, although its effect can be smaller than temperature.	Excessive or insufficient treatment may influence labile carbon and potentially toxic organic compounds.	Wang et al. (2025)
Solid-liquid ratio	Can affect reaction chemistry and product properties, but remains less studied.	May influence process water retention, soluble compounds, and post-treatment need.	Wang et al. (2025); Farru et al. (2022)
Post-treatment after HTC	Washing, aging, co-composting, drying, extraction, or thermal finishing can reduce phytotoxicity.	Post-treatment is often necessary before hydrochar can be used safely in sensitive growing media.	Dalias et al. (2018); Hitzl et al. (2018); Puccini et al. (2018); Roehrdanz et al. (2019)

Hydrochar should not be treated as equivalent to either biochar or peat, even though it may share some functional similarities with both materials (Fornes & Belda, 2018; Farru et al., 2022). Biochar is produced by pyrolysis under dry, oxygen-limited conditions and often has a more alkaline reaction, higher aromaticity, and greater structural stability than hydrochar, depending on feedstock and pyrolysis temperature (Fornes & Belda, 2018; Wang et al., 2025). Hydrochar, by contrast, is produced in water under lower-temperature hydrothermal conditions and may retain higher amounts of labile organic carbon and process-water-derived compounds (Farru et al., 2022; Hitzl et al., 2018).

For hydrochar to be used as a growing medium constituent, its physical, physicochemical, and biological properties must be suitable for containerized root-zone conditions (Fornes & Belda, 2018; Belda et al., 2016). Critical parameters include total porosity, air-filled porosity, water-holding capacity, bulk density, particle size distribution, pH, electrical conductivity, nutrient content, cation exchange behavior, labile carbon content, microbial respiration, and possible phytotoxicity (Fornes & Belda, 2018; Fornes & Belda, 2016; Farru et al., 2022). Table 2 presents the key material properties that determine hydrochar suitability for ornamental growing media.

Table 2. Key material properties that determine hydrochar suitability for ornamental growing media

Property	Possible benefit	Possible limitation	Interpretation for ornamental production
Organic carbon content	May contribute to carbon recycling and substrate organic matter.	Labile carbon may stimulate microbial respiration and oxygen depletion.	Hydrochar should be biologically stabilized or tested before use in containers.
Water-holding capacity	May improve moisture retention, especially in coarse or sandy systems.	Excessive water retention may reduce aeration and root oxygen supply.	Hydrochar proportion should be limited and blended with structurally stable components.
Air-filled porosity	Adequate porosity supports root respiration.	Fine or hygroscopic hydrochar may reduce air space.	Air-water balance must be tested before crop use.
pH	May help adjust acidic or nutrient-poor media depending on hydrochar type.	Unsuitable pH can affect nutrient availability and plant quality.	pH should be corrected before potting or seedling production.
Electrical conductivity	Nutrient-rich hydrochar may supply some elements.	High soluble salts may inhibit germination or young plant growth.	EC screening is essential for ornamental seedlings and sensitive species.
Process water residues	May contain nutrients and soluble organics.	Phenols, furfurals, organic acids, and volatile compounds may cause phytotoxicity.	Washing, aging, co-composting, or other post-treatment may be needed.
Microbial respiration	Can indicate biological activity.	High respiration may lead to CO_2 accumulation and root-zone O_2 depletion.	Hydrochar should not be used fresh at high proportions in poorly aerated media.
Nitrogen dynamics	May interact with nutrient cycling.	Nitrogen immobilization can reduce early plant growth.	N availability should be monitored in hydrochar-containing substrates.

Figure 1 illustrates the overall pathway from feedstock selection and HTC process documentation to post-treatment, quality checkpoints, and validated hydrochar use in ornamental growing media.

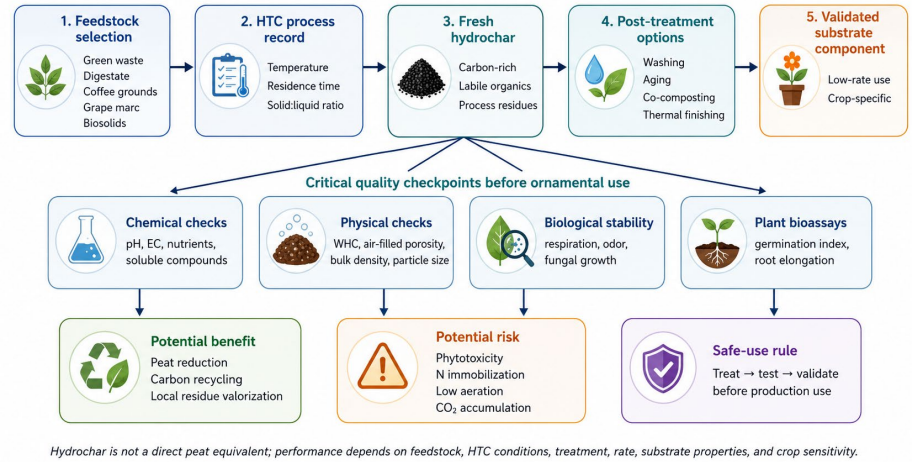

Figure 1. From hydrothermal carbonization to ornamental growing media

2. Hydrochar as a Peat Substitute and Growing Medium Component in Ornamental and Horticultural Production

Hydrochar has been investigated as a potential component of peat-reduced or peat-free growing media because it is carbon-rich, can be produced from regionally available organic residues, and may show peat-like characteristics depending on feedstock and HTC conditions (Farru et al., 2022; Puccini et al., 2018). However, the available evidence indicates that hydrochar should not be considered a direct one-to-one substitute for peat, particularly in ornamental and containerized production systems where substrate physical stability, aeration, water retention, pH, EC, root-zone oxygen, and absence of phytotoxicity are critical quality requirements (Fornes & Belda, 2018; Roehrdanz et al., 2019).

In ornamental plant production, hydrochar use is especially sensitive because crop value is closely linked to visual quality, uniformity, flowering performance, root health, and marketable appearance rather than biomass alone (Fornes & Belda, 2018). The studies reviewed here show that hydrochar performance depends on hydrochar type, feedstock, post-treatment, blending ratio, substrate composition, plant species, and cultivation duration (Fornes & Belda, 2018; Belda et al., 2016; Roehrdanz et al., 2019). Table 3 summarizes the main experimental evidence on hydrochar use in growing media and ornamental or horticultural systems.

Table 3. Experimental evidence on hydrochar use in growing media and ornamental/horticultural systems

Study	Hydrochar source / treatment	Plant or test system	Hydrochar proportion / use	Main finding	Implication for ornamental production
Fornes & Belda (2018)	Forest waste hydrochar compared with forest waste biochar and olive mill waste biochar	Calendula officinalis and Petunia hybrida	Coir-based substrates containing 10%, 25%, and 50% char fractions	Hydrochar substrates retained too much water, were poorly aerated, and showed high CO_2 concentrations; acceptable plant performance was observed only up to 25% hydrochar.	Hydrochar may be used only at limited proportions unless its physical and biological behavior is improved.
Roehrdanz et al. (2019)	Co-composted hydrochar from brewer's spent grains and green waste	Chinese cabbage germination test; Tagetes patula growth trial	10%, 30%, and 50% hydrochar-based co-composted substrates	Co-composting improved germination and plant growth compared with freshly mixed hydrochar substrates; Tagetes quality approached peat-based substrate under some conditions.	Co-composting is a promising strategy for ornamental use, especially for flowering bedding plants.
Belda et al. (2016)	Forest waste hydrochar compared with biochars	Myrtus communis and Pistacia lentiscus	Hydrochar as constituent of soilless substrates / soil blends	Responses differed by species; hydrochar performed better in some soil-based combinations but was not universally beneficial.	Species-specific testing is essential before applying hydrochar in ornamental shrubs.
Fornes & Belda (2016)	Summary of UPV studies on biochar and hydrochar	Ornamental, forestry, vegetable, germination, rooting, and seedling systems	Different substrate combinations	Biochar and hydrochar did not perform well as sole substrates; hydrochar's high hygroscopicity and biological activity may reduce root-zone O_2.	Hydrochar should be evaluated as a blended component rather than a stand-alone substrate.
Neumaier et al. (2017)	Hydrochars from woody landscaping material and woody material plus grass clippings	Potted basil; Chinese cabbage, lettuce, and barley tests	20-100% by volume; basil trial included 30% and 50% hydrochar mixtures	30% hydrochar reduced basil growth; higher proportions nearly stopped post-germination growth; N immobilization and phytotoxic substances were key limitations.	High hydrochar proportions are risky in potted crops unless co-composting or stabilization is used.

Study	Hydrochar source / treatment	Plant or test system	Hydrochar proportion / use	Main finding	Implication for ornamental production
Álvarez et al. (2017)	Hydrochars from biosolids and urban wastes	Lolium perenne	Hydrochar-peat mixtures	Biosolid hydrochar and hydrochar-peat mixtures increased dry matter production compared with peat control.	Some hydrochar-peat blends can perform well, but feedstock safety and crop sensitivity must be evaluated.
Farru et al. (2022)	Hydrochars from digestate, spent coffee grounds, and grape marc; extraction, washing, drying	Cress germination tests	Peat replacement at different rates	All hydrochars had potential to replace at least 5% peat; spent coffee ground and grape marc hydrochars inhibited above 5%, while post-treated digestate hydrochar improved root growth.	Low replacement ratios and post-treatment are critical for safe use in growing media.
Puccini et al. (2018)	Industrial-scale hydrochar from municipal woody and herbaceous prunings; fresh, washed, and aged	Lettuce germination and radicle tests	5% and 10% hydrochar in potting mix	Fresh hydrochar inhibited germination and radicle length; aging was more effective than washing in reducing phytotoxicity.	Fresh hydrochar should not be used directly as a potting mix component without post-treatment.
Eulenstein et al. (2021)	Hydrochar from extensive grassland biomass; abstract-level evidence	Chinese cabbage, impatiens, and daisies	10%, 20%, and 30% hydrochar added to commercial medium	Some hydrochar mixtures produced comparable plant growth, but fungal and odor problems and possible N immobilization were reported.	Supportive only because full text was not accessible; post-processing appears necessary.

The study by Fornes and Belda (2018) is one of the most directly relevant studies for ornamental plant cultivation because it used Calendula officinalis and Petunia hybrida, two ornamental species with different substrate and salinity sensitivities. Their results showed that hydrochar-containing substrates had physical and biological limitations, especially excessive water retention, poor aeration, and high CO_2 accumulation.

Co-composting appears to be one of the most promising approaches for improving hydrochar suitability in horticultural growing media (Roehrdanz et al., 2019; Bona et al., 2023). Roehrdanz et al. (2019) evaluated co-composted hydrochar substrates using Chinese cabbage germination tests and a growth trial with French marigold (Tagetes patula), and showed that co-composting

improved both germination and plant growth compared with untreated hydrochar mixtures.

Belda et al. (2016) evaluated biochars and hydrochars as substrate constituents for the soilless growth of Myrtus communis and Pistacia lentiscus, two Mediterranean woody species relevant to ornamental and landscape uses. The study showed that plant response depended on both species and substrate composition, indicating that hydrochar suitability is not universal even among woody ornamentals.

Across the available ornamental and horticultural evidence, a consistent pattern emerges: hydrochar may be useful as a partial component of growing media, but fresh or high-proportion hydrochar is often risky (Fornes & Belda, 2018; Neumaier et al., 2017; Luutu et al., 2022).

3. Hydrochar Pre- and Post-Treatment Strategies for Improving Growing Media Suitability

The suitability of hydrochar for ornamental and horticultural growing media is strongly influenced by whether the material is used fresh or after an appropriate pre- or post-treatment process (Dalias et al., 2018; Hitzl et al., 2018; Puccini et al., 2018; Roehrdanz et al., 2019). Fresh hydrochar may retain process water, labile organic compounds, volatile organic substances, organic acids, phenols, furans, furfurals, and other soluble or semi-volatile compounds that can inhibit germination, restrict root elongation, or reduce early plant growth (Farru et al., 2022; Hitzl et al., 2018; Karatas et al., 2022).

Pre- and post-treatment strategies are not only technical additions to hydrochar processing; they are central determinants of whether hydrochar behaves as a beneficial substrate component or as a phytotoxic material (Luutu et al., 2022). In their meta-analysis, Luutu et al. (2022) showed that hydrochar modification significantly mitigated negative effects on seed germination and shoot biomass. Table 4 summarizes the main pre- and post-treatment strategies for improving hydrochar suitability in growing media.

Table 4. Pre- and post-treatment strategies for improving hydrochar suitability in growing media

Treatment strategy	Main purpose	Reported effect	Possible limitation	Key sources
Washing / leaching	Remove water-soluble phytotoxic compounds and process water residues.	Can reduce soluble organic compounds and improve germination in some hydrochars.	May not remove all phytotoxic fractions; may also remove useful nutrients; generates wastewater.	Farru et al. (2022); Puccini et al. (2018)
Aging	Allow volatile compounds, labile organics, and unstable fractions to decline over time.	Puccini et al. (2018) found aging more effective than washing for reducing phytotoxicity.	Requires time and storage space; effect depends on feedstock and environmental conditions.	Puccini et al. (2018); Karatas et al. (2022)
Co-composting	Stabilize labile carbon, biologically degrade phytotoxic compounds, and improve nutrient dynamics.	Improved germination and Tagetes patula growth compared with fresh hydrochar mixtures.	Requires controlled composting conditions; results depend on compost and hydrochar properties.	Roehrdanz et al. (2019); Bona et al. (2023)
Aerobic stabilization with compost	Reduce phytotoxicity and improve amendment maturity.	Stabilized hydrochar-compost mixtures improved plant growth compared with compost alone.	High hydrochar doses may still increase microbial activity and root anoxia risk.	Bona et al. (2023)
Thermal finishing treatment	Remove volatile organic compounds associated with phytotoxicity.	Treatment at approximately 275 °C eliminated phytotoxicity from the solid hydrochar.	Requires additional energy and process control; phytotoxicity is transferred to the liquid fraction.	Hitzl et al. (2018)
Drying / extraction	Reduce retained water and selected soluble compounds, depending on feedstock.	Used in hydrochars from digestate, spent coffee grounds, and grape marc to modify germination response.	Effect is feedstock-specific and may be insufficient at higher replacement rates.	Farru et al. (2022)
Plant-based bioassay screening	Verify whether treatment reduced phytotoxicity.	Germination index and root elongation tests detect residual toxicity before crop use.	Bioassays are indicators, not full crop performance tests.	Farru et al. (2022); Hitzl et al. (2018); Luutu et al. (2022)

Washing is one of the simplest post-treatment strategies used to reduce soluble compounds retained in hydrochar after HTC (Dalias et al., 2018; Farru et al., 2022; Puccini et al., 2018). However, washing should not be interpreted as universally sufficient because some phytotoxic compounds may not be fully removed by simple leaching and because washing may generate an additional wastewater stream.

Aging and biological stabilization can reduce hydrochar phytotoxicity by allowing volatile compounds to dissipate, labile organics to transform, microbial communities to develop, and unstable chemical fractions to become less harmful to plants (Puccini et al., 2018; Karatas et al., 2022). Co-composting is particularly promising for horticultural substrates because it can degrade

phytotoxic compounds, stabilize labile carbon, and improve nutrient dynamics (Roehrdanz et al., 2019; Bona et al., 2023).

Thermal finishing treatment offers an alternative to washing, aging, or co-composting when the main objective is to remove volatile organic compounds associated with hydrochar phytotoxicity (Hitzl et al., 2018). Hitzl et al. (2018) reported that treatment at approximately 275 °C eliminated phytotoxicity from the carbonaceous solid, although the phytotoxic property was transferred to the separated liquid fraction.

Table 5. Linking hydrochar treatment methods with practical ornamental growing media risks

Main risk in fresh hydrochar	Treatment option most directly addressing the risk	Practical interpretation for ornamental production
Soluble organic acids, furans, phenols, and process water residues	Washing, extraction, drying, aging	Useful for reducing early germination inhibition, but must be verified by germination index and root elongation tests.
Volatile phytotoxic compounds and odor	Thermal finishing, aging, aeration	Relevant where odor or volatile emissions could affect crop quality or worker acceptance.
Labile carbon and high microbial respiration	Co-composting, aerobic stabilization, aging	Important for preventing CO_2 accumulation, low O_2, root anoxia, and unstable potting media.
Nitrogen immobilization	Co-composting, nutrient adjustment, maturation	Essential where early growth suppression occurs despite successful germination.
Unstable pH and EC	Washing, blending, chemical characterization	Necessary before seedling or sensitive ornamental species production.
Species-specific sensitivity	Crop-specific pot trials	Required because acceptable hydrochar ratios differ among ornamental species and crop stages.
High incorporation rates	Low-rate validation and gradual replacement strategy	Hydrochar should first be tested at low rates before increasing the peat replacement proportion.

4. Phytotoxicity, Nitrogen Immobilization, and Root-Zone Risks

Hydrochar use in ornamental and horticultural growing media is limited primarily by its inconsistent effects on germination, root elongation, early seedling establishment, and shoot development (Luutu et al., 2022; Puccini et al., 2018; Karatas et al., 2022). These limitations do not arise from a single mechanism; rather, they are linked to interacting chemical, biological, and physical processes, including residual organic compounds, process-water-derived phytotoxins, pH and EC imbalance, nitrogen immobilization, high microbial respiration, CO_2 accumulation, and reduced root-zone oxygen availability (Fornes & Belda, 2018; Hitzl et al., 2018; Bona et al., 2023).

Seed germination and root elongation tests are commonly used as early indicators of hydrochar suitability because germinating seeds and young radicles are highly sensitive to soluble phytotoxic compounds, salinity, low oxygen, and pH imbalance (Farru et al., 2022; Puccini et al., 2018; Hitzl et al., 2018). The broader evidence confirms that negative germination responses are not isolated cases: Luutu et al. (2022) reported a 38% average reduction in seed germination across studies.

Fresh hydrochar may contain organic acids, phenolic compounds, furans, furfurals, volatile organic compounds, aldehydes, ketones, and other soluble intermediates retained in process water or on the hydrochar surface (Farru et al., 2022; Hitzl et al., 2018; Karatas et al., 2022). Celletti et al. (2021) also associated manure-based digestate hydrochar phytotoxicity with extract characteristics, pH, and furanic compounds such as furfural and hydroxymethylfurfural.

Nitrogen immobilization is another important non-toxicological mechanism by which hydrochar may reduce plant growth (Neumaier et al., 2017; Luutu et al., 2022). Hydrochar can contain labile carbon fractions that stimulate microbial growth, and microbial communities may immobilize mineral nitrogen during decomposition or stabilization processes.

Table 6. Main hydrochar-related risk mechanisms in ornamental growing media

Risk mechanism	Main cause	Plant response	Relevance for ornamental production	Key sources
Germination inhibition	Soluble phytotoxic compounds, process water residues, high application rate	Reduced germination percentage, delayed emergence	Poor stand uniformity and weak seedling establishment	Farru et al. (2022); Luutu et al. (2022); Puccini et al. (2018)
Root elongation restriction	Organic acids, phenols, furans, furfurals, volatile compounds	Short radicles, poor root branching, weak root systems	Reduced transplant quality and root-zone exploration	Hitzl et al. (2018); Celletti et al. (2021); Karatas et al. (2022)
Osmotic or salt stress	High EC or soluble ions	Reduced water uptake, chlorosis, growth suppression	Sensitive ornamental seedlings may lose visual quality quickly	Fornes & Belda (2018); Puccini et al. (2018)
pH-related nutrient imbalance	Acidic or alkaline hydrochar reaction	Micronutrient imbalance, nutrient deficiency or toxicity	Leaf color and compactness can be affected	Wang et al. (2025); Fornes & Belda (2018)
Nitrogen immobilization	Labile carbon stimulates microbial N demand	Reduced early growth, chlorosis, low shoot biomass	Delayed growth and reduced market uniformity	Neumaier et al. (2017); Luutu et al. (2022)
High microbial respiration	Easily degradable organic compounds	CO_2 accumulation, low O_2, root stress	Pot-grown roots are vulnerable to oxygen limitation	Fornes & Belda (2018); Bona et al. (2023)

Risk mechanism	Main cause	Plant response	Relevance for ornamental production	Key sources
Root-zone anoxia	Excess water retention plus microbial respiration	Root dysfunction, reduced nutrient uptake	High risk in poorly aerated container substrates	Fornes & Belda (2018); Bona et al. (2023)

Figure 2 synthesizes the main hydrochar-induced phytotoxicity and growth-suppression pathways that may operate in ornamental growing media.

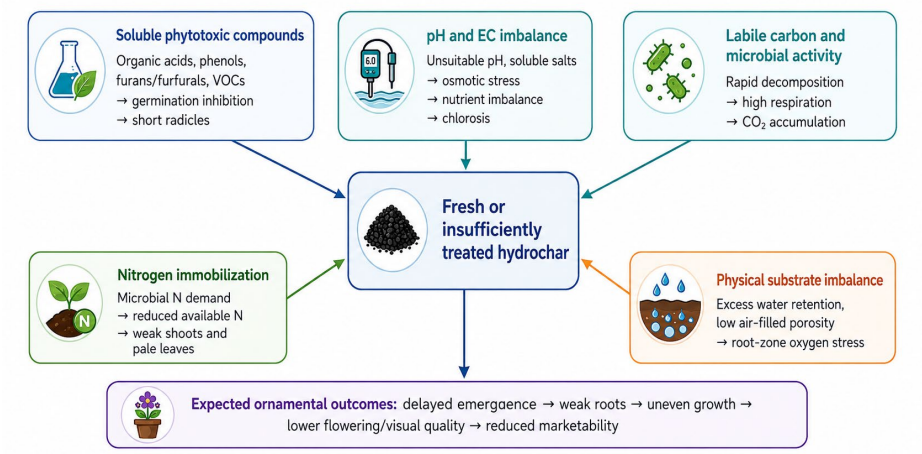

Figure 2. Mechanisms of hydrochar-induced phytotoxicity and growth suppression

5. Application Rate, Mixing Ratio, and Evidence Level

Hydrochar response is strongly dose-dependent, and application rate is one of the most important factors explaining why some studies report growth stimulation while others report germination inhibition or biomass reduction (Luutu et al., 2022). This issue is especially important for ornamental growing media because hydrochar can be expressed either as a soil application dose, such as t ha^{-1}, or as a substrate mixing ratio, such as % v/v or % w/w.

Several growing media studies suggest that low hydrochar proportions are more defensible as a first testing range, particularly when the objective is partial peat replacement rather than full substrate substitution (Farru et al., 2022; Fornes & Belda, 2018). Farru et al. (2022) found that hydrochars produced from digestate, spent coffee grounds, and grape marc showed potential to replace at least 5% peat, although spent coffee ground and grape marc hydrochars caused inhibition above 5%.

Higher hydrochar proportions may increase the probability of growth suppression, especially when hydrochar is fresh or not biologically stabilized (Neumaier et al., 2017; Luutu et al., 2022). Neumaier et al. (2017) reported that hydrochars used as peat substitutes in organically grown potted herbs significantly reduced basil growth even at 30% by volume, while higher proportions nearly stopped post-germination development.

A major challenge in interpreting hydrochar literature is the difference between soil amendment trials and growing medium trials (Luutu et al., 2022; Fornes & Belda, 2018). Soil studies often report hydrochar application in t ha^{-1} or % w/w, whereas substrate studies frequently report hydrochar as % v/v replacement of peat, coir, compost, or other growing medium components.

Table 7. Hydrochar rates and reported plant responses in selected studies

Study	System	Hydrochar rate or proportion	Plant/test species	Reported response	Interpretation for ornamental/growing media use
Luutu et al. (2022)	Meta-analysis of soil-applied hydrochar studies	Variable rates across 43 articles and 437 comparisons	Multiple crops and germination tests	Hydrochar reduced seed germination by 38% and shoot biomass by 10% on average; negative effects increased at higher rates; post-treatment mitigated negative effects.	Rate control and post-treatment are central to safe use.
Farru et al. (2022)	Peat replacement / germination test	At least 5% peat replacement; higher rates tested depending on hydrochar	Cress	All hydrochars showed potential to replace at least 5% peat; some feedstocks caused inhibition above 5%.	5% is a reasonable initial screening level, but feedstock-specific testing is required.
Fornes & Belda (2018)	Coir-based ornamental substrates	10%, 25%, and 50% char fractions	Calendula officinalis and Petunia hybrida	Plant performance was acceptable only up to 25% forest waste biochar or hydrochar; hydrochar caused excessive water retention and poor aeration.	Hydrochar can be tested at moderate rates only if air-water balance is suitable.

Study	System	Hydrochar rate or proportion	Plant/test species	Reported response	Interpretation for ornamental/growing media use
Neumaier et al. (2017)	Peat substitute in potted herbs	20-100% v/v hydrochar	Basil and supporting bioassays	30% hydrochar significantly reduced basil growth; higher proportions nearly stopped growth after germination.	High-volume replacement is risky without co-composting or stabilization.
Roehrdanz et al. (2019)	Co-composted hydrochar substrate	10%, 30%, and 50% hydrochar-based mixtures	Chinese cabbage, Tagetes patula	Co-composting improved germination and growth; some Tagetes responses approached peat-based substrate quality.	Higher hydrochar proportions may be feasible only after co-composting and validation.
Puccini et al. (2018)	Potting mix germination test	5% and 10% hydrochar	Lettuce	Fresh hydrochar inhibited germination and radicle length; aging improved safety more than washing.	Even low fresh hydrochar rates may be phytotoxic.
Álvarez et al. (2017)	Hydrochar-peat mixtures	Hydrochar and 50% v/v hydrochar-peat mixtures	Lolium perenne	Biosolid hydrochar and hydrochar-peat mixtures increased dry matter production compared with peat control.	Some hydrochar-peat blends can perform well, but feedstock and crop context matter.

Table 8. Proposed rate-validation framework for hydrochar use in ornamental growing media

Validation stage	Suggested hydrochar level	Main tests	Decision criterion	Interpretation
Screening control	0%	Standard commercial or peat/coir-based medium	Baseline plant and substrate performance	Required for comparison.
Initial low-rate test	5%	pH, EC, germination index, root elongation, early seedling growth	No inhibition compared with control	Suitable for cautious further testing.
Moderate low-rate test	10%	Substrate physical properties, seedling vigor, leaf color, root growth	No reduction in emergence, root length, or early quality	May be considered for pot trial continuation.
Conditional medium-rate test	20-25%	Air-filled porosity, water retention, CO_2/respiration indicators, crop pot trial	Acceptable root-zone aeration and plant uniformity	Only for treated or stable hydrochar.

Validation stage	Suggested hydrochar level	Main tests	Decision criterion	Interpretation
High-risk rate test	≥30%	Full crop trial, nutrient dynamics, N immobilization, root health, visual quality	No growth delay, chlorosis, odor, fungal growth, or root restriction	Not recommended without strong treatment and crop-specific evidence.
Commercial validation	Final selected rate	Multi-cycle or production-scale trial	Stable quality under real irrigation/fertilization conditions	Required before grower recommendation.

Figure 3 presents a stepwise rate-validation pathway for increasing hydrochar use only after chemical, physical, biological, and plant-quality checkpoints are passed.

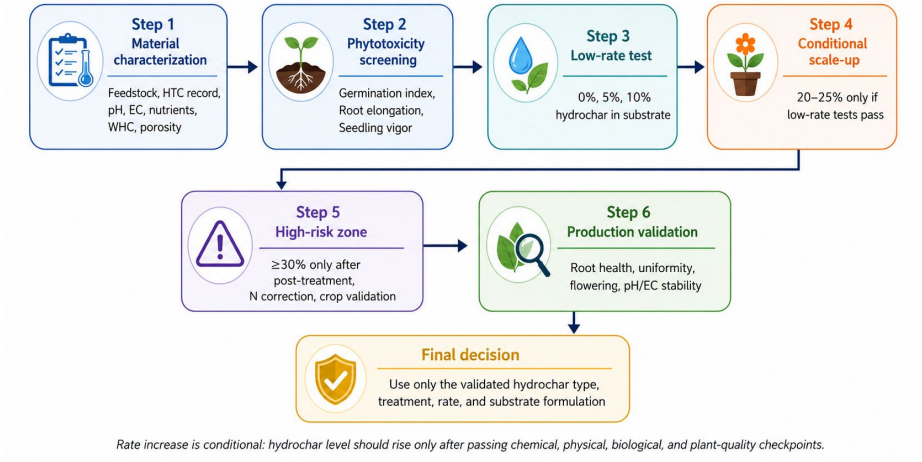

Figure 3. Evidence-based hydrochar rate validation pathway for ornamental growing media

6. Soil Amendment Functions of Hydrochar and Their Transferability to Ornamental Plant Systems

Hydrochar has been studied not only as a peat substitute or growing medium constituent, but also as a soil amendment for improving soil organic carbon, nutrient cycling, water retention, aggregation, and biological activity (Wang et al., 2025; Bona et al., 2023). However, the interpretation of soil amendment studies requires caution when transferring conclusions to ornamental plant production because open-field soils and containerized growing media differ strongly in buffering capacity, root-zone volume, aeration, drainage, microbial dynamics, and nutrient management (Fornes & Belda, 2018; Luutu et al., 2022).

Hydrochar is a carbon-rich material that can contribute organic carbon to soil, although its stability and biological behavior vary with feedstock, HTC temperature, residence time, and post-treatment (Wang et al., 2025; Bona et al., 2023). Hydrochar can also affect soil pH and nutrient availability, but the direction and magnitude of these effects depend on feedstock and process conditions (Wang et al., 2025).

One of the most frequently discussed benefits of hydrochar is its potential to improve soil water-holding capacity, particularly in sandy or coarse-textured soils (Wang et al., 2025). This function may be valuable for landscape ornamentals, urban green spaces, nursery fields, and open-field ornamental crops grown under water-limited conditions.

Hydrochar can influence soil biological processes, including arbuscular mycorrhizal colonization, nodulation, and soil aggregation, but reported responses are not uniform (Rillig et al., 2010; George et al., 2012). Rillig et al. (2010) reported that higher additions could negatively affect plant growth, while arbuscular mycorrhizal colonization and fungal spore germination showed stimulatory responses under some conditions. George et al. (2012) reported positive effects on soil aggregation but divergent effects on mycorrhizal colonization and nodulation.

Table 9. Soil amendment functions of hydrochar and their relevance for ornamental plant systems

Hydrochar function	Possible positive effect in soil	Possible risk or limitation	Transferability to ornamental systems	Main sources
Organic carbon input	May increase soil organic carbon and support carbon recycling.	Labile carbon may stimulate excessive microbial respiration or instability.	Relevant for nursery soils and landscape soils, but less predictable in containers.	Wang et al. (2025); Bona et al. (2023)
Nutrient contribution	May supply macro- and micronutrients depending on feedstock.	Nutrient release may be unbalanced; N immobilization may reduce early growth.	Useful only after nutrient analysis and crop-specific fertilization adjustment.	Wang et al. (2025); Luutu et al. (2022)
Water retention	May improve water availability in sandy or coarse soils.	Excess water retention may reduce aeration in pots.	Potentially useful for landscape soils; risky in fine or poorly aerated substrates.	Wang et al. (2025); Fornes & Belda (2018)
Soil aggregation	May improve soil structure under some conditions.	Response may depend on hydrochar chemistry and soluble fractions.	Relevant for open-field nursery soils and landscape establishment.	George et al. (2012)
Mycorrhizal interaction	May stimulate some fungal responses under certain conditions.	May suppress mycorrhizal colonization depending on hydrochar and leachate.	Needs testing for mycorrhiza-dependent ornamental species.	Rillig et al. (2010); George et al. (2012)

Hydrochar function	Possible positive effect in soil	Possible risk or limitation	Transferability to ornamental systems	Main sources
Biological activity	May enhance microbial processes and organic matter transformation.	Excess respiration may cause O_2 depletion and root stress.	More manageable in soil than in containers; requires stabilization.	Bona et al. (2023); Fornes & Belda (2018)
Waste valorization	Converts wet organic residues into potentially useful soil products.	Feedstock contaminants and process-derived compounds must be controlled.	Relevant for circular horticulture and local substrate production.	Hitzl et al. (2018); Puccini et al. (2018)

Table 10. Comparison of hydrochar evaluation criteria for soil amendment and peat replacement uses

Evaluation criterion	Soil amendment use	Peat replacement / growing medium use
Main purpose	Improve soil organic carbon, water retention, nutrient cycling, or structure.	Replace part of peat/coir and function as a root-zone substrate component.
Exposure environment	Diluted in mineral soil with buffering capacity.	Confined container root zone with limited buffering.
Key physical concern	Soil structure, infiltration, water retention, aggregation.	Air-filled porosity, water retention, bulk density, shrinkage, compaction.
Key chemical concern	pH, EC, nutrient release, contaminants, long-term stability.	pH, EC, soluble phytotoxins, nutrient balance, young-root sensitivity.
Key biological concern	Soil microbial activity, mycorrhiza, decomposition, N cycling.	Excess respiration, CO_2 accumulation, N immobilization, root-zone oxygen.
Suitable testing scale	Soil incubation, pot/field trial, amendment-rate experiment.	Germination test, root elongation test, substrate analysis, crop pot trial.
Main risk	Over-application, contaminants, phytotoxicity, nutrient imbalance.	Germination inhibition, root restriction, poor aeration, visual quality loss.
Ornamental relevance	Landscape soils, nursery fields, urban greening, open-field ornamentals.	Bedding plants, potted ornamentals, seedling production, propagation media.

Figure 4 distinguishes the two major application pathways of hydrochar in ornamental systems: soil amendment use and peat replacement/growing medium use.

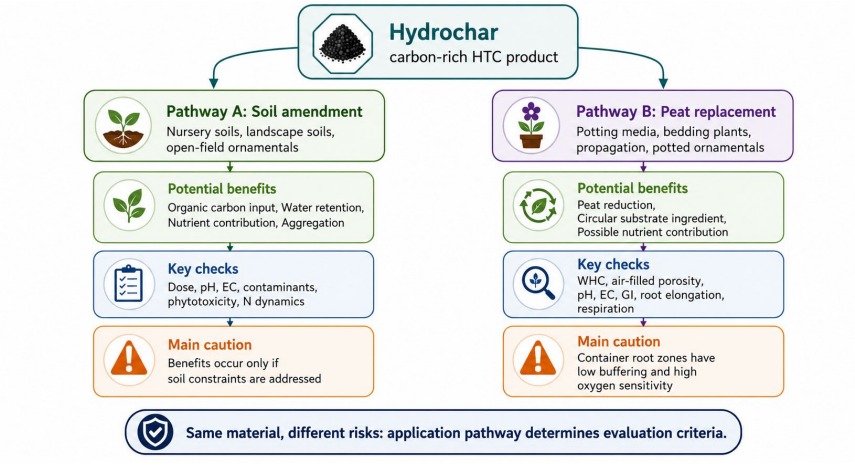

Figure 4. Two application pathways of hydrochar in ornamental plant systems

7. Safe-Use Framework and Decision Flow for Hydrochar in Ornamental Production

The available evidence indicates that hydrochar should not be introduced into ornamental plant production as a raw, untested, or universally safe peat substitute. Its use requires a structured validation framework because plant response is controlled by feedstock origin, HTC conditions, post-treatment, application rate, substrate formulation, and crop sensitivity (Luutu et al., 2022; Wang et al., 2025).

A safe-use framework should combine material traceability, physicochemical characterization, phytotoxicity screening, post-treatment selection, low-rate substrate testing, and crop-specific validation. This approach is supported by studies showing that fresh hydrochar may contain phytotoxic organic compounds, that post-treatment can reduce negative plant responses, and that higher application rates increase the probability of germination inhibition or shoot biomass reduction (Hitzl et al., 2018; Karatas et al., 2022; Luutu et al., 2022).

The first step in hydrochar evaluation should be full documentation of the feedstock and HTC production conditions. Feedstock type strongly affects hydrochar nutrient content, ash concentration, labile organic compounds, pH, EC, and potential contaminant profile (Wang et al., 2025; Farru et al., 2022). Before any plant trial, hydrochar and hydrochar-containing substrates should also be analyzed for pH, electrical conductivity, nutrient composition, bulk density, particle size, water-holding capacity, air-filled porosity, and possible indicators of labile organic matter or respiration activity (Fornes & Belda, 2018; Farru et al., 2022).

Table 11. Safe-use checklist for hydrochar in ornamental plant production

Step	Evaluation criterion	Minimum information or test	Decision point	Main supporting sources
1	Feedstock traceability	Biomass type, origin, contamination risk, moisture status	Reject or restrict hydrochar if feedstock is unknown or high risk	Wang et al. (2025); Farru et al. (2022)
2	HTC process record	Temperature, residence time, solid-liquid ratio, process scale	Compare only hydrochars with documented production history	Wang et al. (2025); Puccini et al. (2018)
3	Basic chemical screening	pH, EC, nutrients, ash, possible contaminants	Correct, blend, or reject if pH/EC is unsuitable	Fornes & Belda (2018); Farru et al. (2022)

Step	Evaluation criterion	Minimum information or test	Decision point	Main supporting sources
4	Physical substrate testing	WHC, air-filled porosity, bulk density, particle size	Continue only if water-air balance is suitable	Fornes & Belda (2018)
5	Phytotoxicity test	Germination index, radicle length, seedling vigor	Post-treat or reject if inhibition is observed	Hitzl et al. (2018); Puccini et al. (2018); Farru et al. (2022)
6	Post-treatment	Washing, aging, co-composting, aerobic stabilization, thermal finishing	Select treatment according to risk mechanism	Dalias et al. (2018); Roehrdanz et al. (2019); Bona et al. (2023)
7	Low-rate substrate trial	0%, 5%, 10% hydrochar replacement	Move upward only if crop and substrate indicators remain stable	Farru et al. (2022); Luutu et al. (2022)
8	Crop-specific validation	Target ornamental species, root quality, leaf color, uniformity, flowering	Use only if ornamental quality is maintained	Fornes & Belda (2018); Roehrdanz et al. (2019)
9	Production monitoring	EC, pH, N availability, odor, fungal growth, root health	Adjust fertilization or reduce hydrochar rate if problems occur	Neumaier et al. (2017); Bona et al. (2023)

Table 12. Decision criteria for accepting, treating, or rejecting hydrochar for ornamental media

Observation	Interpretation	Recommended decision
Unknown feedstock or undocumented HTC process	Quality and contamination risk cannot be assessed	Do not use for ornamental media until documentation is available.
High EC or unsuitable pH	Risk of osmotic stress or nutrient imbalance	Blend, wash, correct chemically, or reject depending on severity.
Germination inhibition or short radicles	Residual phytotoxicity likely	Apply post-treatment and retest; do not proceed directly to crop trial.
High water retention and low air-filled porosity	Root-zone oxygen risk	Reduce hydrochar rate or blend with coarse structural materials.
Odor, fungal growth, or high respiration	Biological instability or labile carbon	Age, aerate, co-compost, or stabilize before use.
Early chlorosis or weak shoot growth	Possible N immobilization or nutrient imbalance	Check N availability and fertilization; consider composting or lower rate.
Acceptable germination but poor post-germination growth	Bioassay insufficient; root-zone or nutrient problem likely	Conduct full crop pot trial before recommendation.
Stable growth, good roots, no odor, acceptable pH/EC	Hydrochar may be suitable at tested rate	Continue crop-specific validation and production-scale testing.
Good performance in one species only	Species-specific suitability	Do not generalize to other ornamental crops without testing.

Figure 5 provides a safe-use decision flow that integrates documentation, characterization, bioassay screening, post-treatment, low-rate trials, crop validation, and production monitoring.

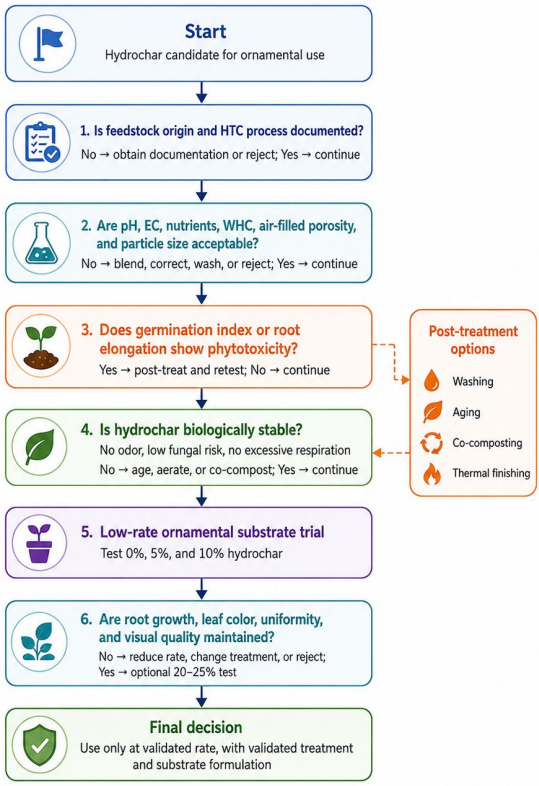

Figure 5. Safe-use decision flow for hydrochar in ornamental growing media

CONCLUSION

Hydrochar is a promising but highly variable hydrothermal carbonization product with potential relevance for agricultural production, ornamental plant cultivation, peat reduction, and circular use of wet organic residues. Its main advantage is associated with the possibility of converting high-moisture biomass into a carbon-rich material without the intensive pre-drying required for pyrolysis, which makes hydrochar particularly relevant for digestates, green wastes, municipal residues, food wastes, spent coffee grounds, grape marc, and other organic waste streams (Farru et al., 2022; Puccini et al., 2018; Wang et al., 2025).

The available evidence does not support the direct and generalized use of fresh hydrochar as a peat substitute in ornamental growing media. Studies with ornamental and horticultural crops show that hydrochar performance is strongly influenced by feedstock type, HTC conditions, post-treatment, mixing

ratio, substrate formulation, and plant species (Fornes & Belda, 2018; Roehrdanz et al., 2019; Belda et al., 2016).

A central conclusion of this review is that hydrochar should be considered a conditional substrate component, not a universal peat replacement. Low incorporation rates, especially around 5-10%, appear more defensible as an initial testing range, but even low rates may be risky when hydrochar is fresh or insufficiently stabilized (Farru et al., 2022; Puccini et al., 2018). Moderate rates around 20-25% may be possible for selected treated or blended hydrochars, whereas rates of 30% and above should be approached with caution unless the material has been post-treated and validated in crop-specific trials (Fornes & Belda, 2018; Neumaier et al., 2017).

Post-treatment is one of the most important steps for improving hydrochar suitability. Washing, aging, co-composting, aerobic stabilization, drying, extraction, and thermal finishing can reduce phytotoxicity or improve biological stability, but their effectiveness depends on hydrochar origin and target use (Dalias et al., 2018; Hitzl et al., 2018; Roehrdanz et al., 2019; Bona et al., 2023).

For soil amendment use, hydrochar may provide benefits related to organic carbon input, nutrient contribution, water retention, and soil structural improvement, particularly in coarse-textured or degraded soils (Wang et al., 2025). Yet these soil-based benefits should not be transferred directly to containerized ornamental substrates. Therefore, hydrochar should be evaluated through two distinct pathways: as a soil amendment for nursery, landscape, and open-field ornamental systems, and as a partial growing medium component for containerized production.

Overall, hydrochar has genuine potential in ornamental plant production, but this potential can be realized only through controlled processing, post-treatment, low-rate blending, and plant-based validation. Future research should focus on species-specific responses, standardized substrate testing protocols, long-term effects on root-zone biology, nitrogen dynamics, air-water balance, odor and fungal risks, and production-scale trials with economically important ornamental crops.

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CHAPTER 14

SOIL SCIENCE IN TRANSFORMATION: A REVIEW OF PARADIGM SHIFTS AND THE EMERGING TRANSDISCIPLINARY APPROACH

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INTRODUCTION

Soil science encompasses pedology - the study of soil genesis, morphology, and classification - together with edaphology and related physical, chemical, biological, and applied domains (Weil & Brady, 2017). Its roots extend to some of humanity's oldest forms of practical environmental knowledge, whereas its consolidation as an independent scientific discipline occurred in the late nineteenth century, especially through the work of Vasily Dokuchaev (Brevik & Hartemink, 2010; Dokuchaev, 1883). Earlier European scientific accounts often emphasized geological weathering and parent material, although agricultural chemistry, natural history, and practical soil knowledge also contributed to the field's development (Brevik & Hartemink, 2010). Dokuchaev established soil as an independent natural body formed through interactions among climate, organisms, parent material, topography, and time; Jenny later formalized these controls as state factors (Jenny, 1941). This perspective supported the development of distinct research methods and classification systems (Buol et al., 2011; Soil Survey Staff, 1999).

Review scope and approach. This chapter adopts a critical narrative review rather than a systematic review or meta-analysis. The cited literature was selected for its historical, conceptual, or thematic relevance to the proposed transitions. Here, paradigm shift is used operationally to describe a broad reorientation in the dominant questions, concepts, methods, and societal roles of soil science. The four stages overlap and accumulate; they should not be read as mutually exclusive periods in which one perspective completely displaced the preceding one.

Table 1. Summary of four overlapping paradigm transitions in soil science

Paradigm transition	Approximate period	Core perspective	Key limitation / emerging challenge
First (foundation)	Late 19th - mid-20th century	Soil as an independent natural body; pedogenesis and classification	Human influence not explicit; limited resolution of biological complexity
Second (living-system and environmental awakening)	Mid-late 20th century	Soil as a living habitat and an environmental medium for contaminant storage, transformation, and transport	Molecular resolution and integration across scales remained limited

Paradigm transition	Approximate period	Core perspective	Key limitation / emerging challenge
Third (ecosystem services, climate, and health)	Late 20th - early 21st century	Soil as a provider of ecosystem services, climate regulator, biodiversity reservoir, and determinant of health	Governance and social dimensions remained incompletely integrated
Fourth (digital and transdisciplinary)	Emerging 21st century	Soil as a biophysical and social-technical system; digital, ethical, and participatory dimensions	Need for co-production, equity, data governance, uncertainty reporting, and explainability

Source: Authors' synthesis based on Brevik and Hartemink (2010), Richter (2007), Lehmann et al. (2020), and Lang et al. (2012).

Since its consolidation as a discipline, soil science has repeatedly expanded its questions, methods, and societal relevance (Richter, 2007). This review organizes that development into four overlapping transitions: the establishment of soil as an independent natural body; the recognition of soil as a living and environmental system; the ecosystem-services, climate, and health turn; and an emerging digital and transdisciplinary orientation (Amundson et al., 2015; Lehmann et al., 2020). Table 1 summarizes these transitions, and Figure 1 presents their approximate chronology.

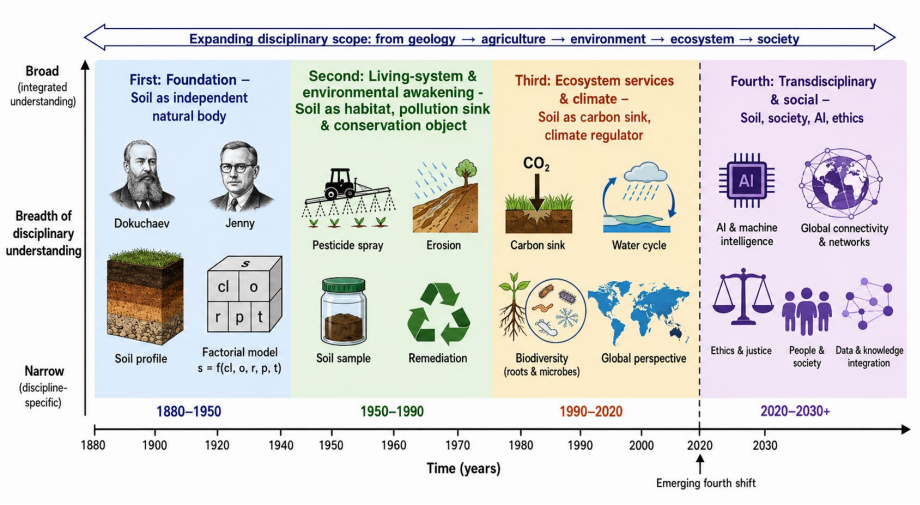

Figure 1. Timeline of four overlapping transitions in soil science (1880-present)
Source: Authors' original conceptual synthesis based on Dokuchaev (1883), Jenny (1941), Alloway (2013), Lal (2015), Ma et al. (2019), and Lehmann et al. (2020).

1.1. First paradigm shift (foundation)

The first transition established soil as an independent scientific object rather than merely a subordinate topic within geology. Through the work of Dokuchaev, Jenny, and others, pedogenesis and genetic classification gained a coherent conceptual foundation (Dokuchaev, 1883; Jenny, 1941). This natural-body framework was transformative, although mainstream pedology initially gave limited explicit attention to cumulative human influence and lacked the tools needed to resolve the internal complexity of microbial communities and soil food webs (Fierer, 2017; Richter, 2007; Young & Crawford, 2004).

1.2. Second paradigm shift (living-system and environmental awakening)

During the mid-to-late twentieth century, two closely related developments broadened soil science. Advances in soil ecology and microstructural analysis strengthened the view of soil as a biologically active habitat, while the environmental movement - prominently influenced by Rachel Carson's Silent Spring - focused attention on soil contamination, conservation, and remediation (Carson, 1962; Young & Crawford, 2004). Soil was therefore increasingly studied not only as a medium for agricultural production but also as a living environmental matrix in which pesticides, trace metals, and industrial contaminants can accumulate, transform, and move (Alloway, 2013; Pepper et al., 2014). This combined biological and environmental awakening linked soil science more closely with ecology, environmental engineering, hydrogeology, ecotoxicology, and public health.

1.3. Third paradigm shift (ecosystem services and climate change)

From the late twentieth century into the early twenty-first century, soil science entered a third broad transition (Wall et al., 2012). Soil came to be understood as a provider of ecosystem services, including carbon storage, water regulation, nutrient cycling, primary production, and habitat for biodiversity (Adhikari & Hartemink, 2016; Lal, 2015). At the same time, the bidirectional relationship between soil and climate became increasingly clear: climate change alters soil temperature, moisture, organic-matter dynamics, and biological activity, while soils can act as sources or sinks of CO_2, N_2O, and CH_4 (Davidson & Janssens, 2006; Paustian et al., 2016). This evidence increased the

prominence of soils in climate-mitigation and sustainable-land-management discussions, while also drawing attention to trade-offs such as N_2O emissions associated with some carbon-sequestration practices (Guenet et al., 2021).

1.4. Fourth paradigm shift (transdisciplinarity and social justice)

The fourth transition proposed in this review concerns the integration of soil's biophysical properties with the social, institutional, cultural, and technological settings in which soil knowledge is produced and used. Land-use decisions are shaped not only by soil properties, climate, and topography but also by tenure, rights, economic interests, historical legacies, cultural values, migration, urbanization, and food-system dynamics (Montgomery, 2007; Richter, 2007). Accordingly, soil science increasingly interacts with the social sciences and humanities without ceasing to be grounded in natural science.

Furthermore, rapidly developing technologies such as artificial intelligence (AI), remote sensing, big data analytics, the Internet of Things (IoT), and digital twins offer new opportunities for soil science. At the same time, they raise ethical, legal, and social issues related to the ownership, privacy, security, and accessibility of soil data (the digital divide), as well as the transparency (interpretability) of AI models (Padarian et al., 2019; Klerkx et al., 2019; Bronson, 2019). The digital divide - the limited access of small and medium-scale farmers, especially in developing countries, to these technologies - points to a problem of technology justice. In addition, the “black box” nature of some AI models creates a methodological tension with the long-standing deterministic, mechanistic tradition in soil science, leading to epistemological discussions.

The following sections examine the historical foundations, contemporary challenges, and future directions of this transformation. The central argument is not that soil science should abandon its natural-science core, but that complex soil problems require collaboration among soil physicists, chemists, biologists, ecologists, geologists, agricultural engineers, planners, economists, sociologists, legal scholars, historians, public-health experts, and affected communities. In this formulation, the discipline's mission expands from understanding and classifying soils to co-producing knowledge that supports sustainable and equitable soil governance.

2. FIRST PARADIGM: SOIL AS AN INDEPENDENT NATURAL BODY

Before Dokuchaev, influential strands of European soil inquiry commonly emphasized geological weathering, mineral composition, and parent material. This was not a single universal view: agricultural chemistry, natural history, and practical traditions had already generated important knowledge about fertility, organisms, and soil variability (Brevik & Hartemink, 2010; Weil & Brady, 2017). What remained absent was a widely accepted, unified theory that treated soil as an independent natural body whose properties emerge from interacting environmental factors.

Dokuchaev fundamentally reorganized this field of inquiry. Through systematic observations of Russian chernozems, he demonstrated that soil is not merely a residue of rock weathering but an independent natural body formed through the interaction of climate, organisms, parent material, topography, and time (Dokuchaev, 1883). Table 2 contrasts a selected pre-Dokuchaev geological emphasis with the Dokuchaevian natural-body framework. Jenny later formalized the soil-forming factors in the state-factor equation:

$$s = f(cl, o, r, p, t, \ldots)$$

where s represents soil properties, and cl, o, r, p, t stand for climate, organisms, parent material, topography, and time, respectively (Jenny, 1941). Figure 2 illustrates this factorial model, showing the interactive relationships among the five factors and their collective influence on soil formation. The equation and diagram became foundational concepts in pedology, providing a systematic framework for studying soil genesis and for developing soil classification systems such as Soil Taxonomy (Soil Survey Staff, 1999; Buol et al., 2011).

Table 2. Comparison of a selected pre-Dokuchaev geological emphasis and the Dokuchaevian natural-body paradigm

Criterion	Selected earlier geological emphasis	Dokuchaevian paradigm
Definition of soil	Primarily weathered mineral material; views varied among traditions	Independent, dynamic natural body
Formation controls	Parent material and geological weathering emphasized	Climate, organisms, parent material, topography, and time interact
Role of organisms	Recognized unevenly and not integrated into a unified genetic model	Organisms included as a state factor; internal complexity not yet resolved
Temporal perspective	Weathering and agricultural timescales, depending on tradition	Pedogenesis across nested timescales
Disciplinary setting	Geology, agricultural chemistry, natural history, and practical agriculture	Autonomous soil-science discipline
Key methods	Geological description, chemical analysis, texture, and mineralogy	Profile description, horizonation, field comparison, and factor-based analysis
Classification	Often linked to parent material or agricultural properties	Based on observed morphological and genetic properties
Main contribution	Accumulated observations of soil variability, minerals, and fertility	Established a coherent natural-body theory of soil
Limitation	No unified factorial theory; biology and human influence represented unevenly	Human influence not explicit; biological mechanisms underresolved

Source: Authors' synthesis based on Brevik and Hartemink (2010), Dokuchaev (1883), Jenny (1941), and Richter (2007).

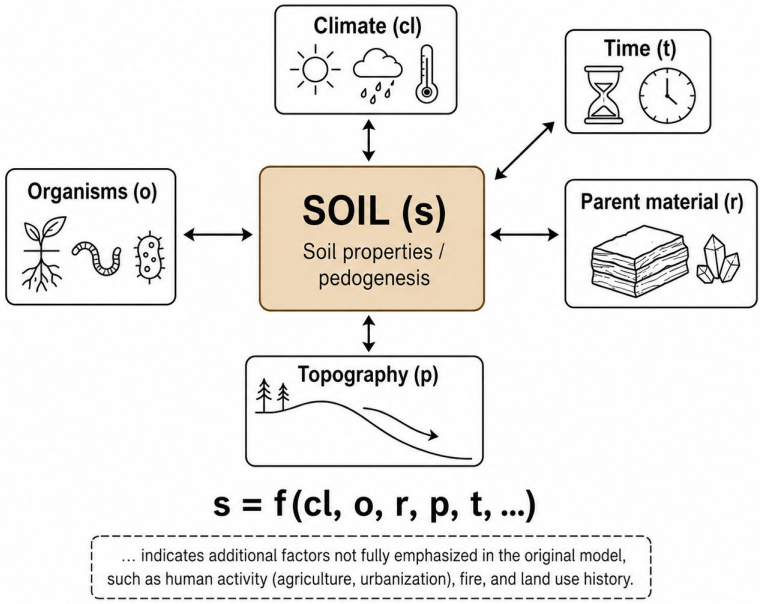

Figure 2. Jenny's factorial model of soil formation: s = f(cl, o, r, p, t, ...)

Source: Authors' original conceptual synthesis based on Jenny (1941).

Despite its revolutionary impact, the classical Dokuchaev-Jenny framework did not explicitly represent the cumulative and sometimes rapid effects of human activity on soil formation (Richter, 2007). Tillage, manuring, irrigation, terracing, mining, and urbanization have altered soils for centuries or millennia, yet humans were not identified as a separate state factor. Organisms were included, but the internal organization of microbial communities, faunal interactions, soil food webs, and root-microbe processes could not be resolved with the methods then available (Fierer, 2017; Young & Crawford, 2004). These are limitations of the framework's explicit scope and historical instrumentation rather than evidence that early soil scientists regarded biology as wholly irrelevant.

The natural-body framework nevertheless established soil science as an autonomous discipline with distinct objects of study, field methods, and classification principles. Its unresolved biological and anthropogenic dimensions later became central to the second transition.

3. SECOND PARADIGM: SOIL AS A LIVING AND ENVIRONMENTAL SYSTEM

Recognition of soil as biologically active predates modern molecular techniques, but developments in microscopy, soil ecology, biochemistry, and later DNA-based methods greatly expanded the resolution at which biological processes could be studied (Brevik & Hartemink, 2010; Fierer, 2017). The second transition therefore did not begin at a single date. It progressively reframed soil as both a structured living habitat and an environmental medium whose biological, chemical, and physical processes are inseparable (Young & Crawford, 2004).

Microscopy and microstructural research revealed nano- and microscale pore networks, clay surfaces, root-soil contacts, bacterial cells, and fungal hyphae within soil aggregates (Young & Crawford, 2004). These observations strengthened the concept of soil as a structured, porous habitat in which water, gases, nutrients, minerals, roots, and microorganisms interact across scales (Tisdall & Oades, 1982). Soil structure was consequently understood not only as a physical arrangement of particles but also as a product and regulator of biological activity.

From the late twentieth century onward, and especially during the twenty-first-century expansion of high-throughput sequencing, molecular methods transformed soil microbiology (Fierer, 2017). Direct extraction and analysis of nucleic acids made it possible to characterize bacterial, fungal, archaeal, protistan, and viral communities without depending exclusively on cultivation (Fierer & Jackson, 2006). Functional genes and metabolic potential could also be investigated, although the presence of a gene does not by itself demonstrate in-situ activity. These methods confirmed soil as one of Earth's most diverse microbial habitats. Figure 3 represents a soil aggregate as a spatially heterogeneous microbial habitat.

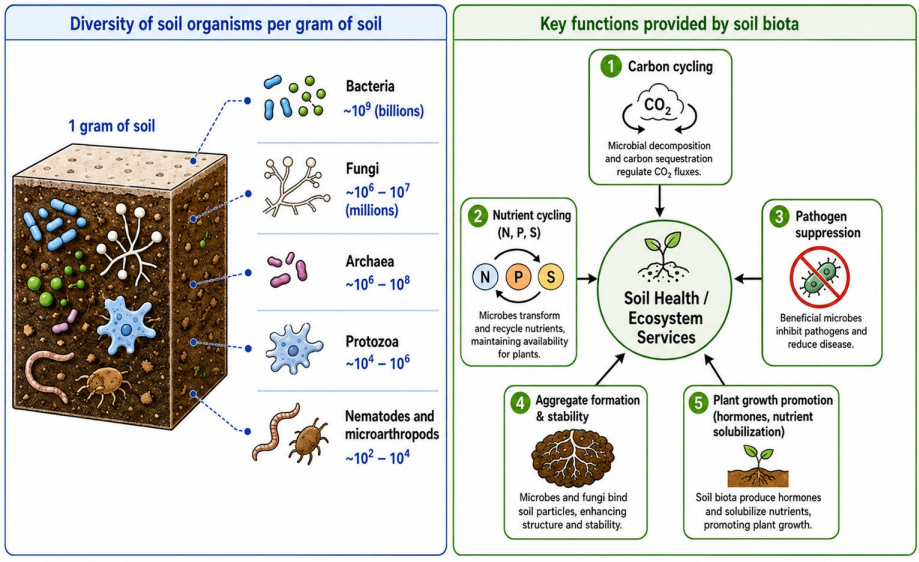

Figure 3. The soil microbiome: diversity and functions in a living system

Source: Authors' original conceptual synthesis based on Fierer (2017) and Jansson and Hofmockel (2020).

These developments contributed to the modern soil-health framework (Doran & Zeiss, 2000). Soil health emphasizes the continuing capacity of soil to function as a living system that supports plants, animals, humans, and ecosystem processes (Lehmann et al., 2020). The terms soil health and soil quality overlap substantially and remain partly contested; soil health often places greater rhetorical and analytical emphasis on biology, resilience, and temporal dynamics, whereas soil quality has frequently been evaluated in relation to fitness for specified functions (Karlen et al., 2019). They should not, however, be treated as a strict dynamic-versus-static dichotomy.

In parallel, environmental research demonstrated that soils store, transform, filter, and transport contaminants. Pesticides, trace metals, industrial wastes, and pathogens interact with mineral surfaces, organic matter, water flow, and soil organisms (Alloway, 2013; Pepper et al., 2014). The second paradigm is therefore defined here as a combined living-system and environmental transition: biological complexity and environmental protection became central rather than peripheral concerns.

4. THIRD PARADIGM: ECOSYSTEM SERVICES, CLIMATE CHANGE, AND ONE HEALTH

The third transition is characterized by three interrelated developments: (i) the framing of soil as a provider of ecosystem services; (ii) recognition of the feedbacks between soil and climate; and (iii) growing attention to soil's direct and indirect relationships with human, animal, plant, and environmental health (Amundson et al., 2015; Wall et al., 2012). Recent work also links Soil Security and One Health through the shared concern for soil condition, ecosystem functioning, and human well-being (Swan et al., 2024). Table 3 summarizes the principal service categories. These functions connect soil science to Sustainable Development Goals concerned with food, water, climate, biodiversity, and land management (Keesstra et al., 2016).

Table 3. Ecosystem services provided by soils and their relevance to human well-being

Service category	Description	Specific examples	Relevance to human well-being
Regulating	Soil controls fluxes of water, carbon, nutrients, and greenhouse gases	Carbon sequestration, water purification, flood regulation, $N_2O/CO_2/CH_4$ exchange	Climate mitigation, water security, air quality
Provisioning	Soil supplies tangible goods	Food (>95% of global production depends directly or indirectly on soils), fiber, biomass, raw materials, and microbial products	Food security, nutrition, energy, medicine
Supporting	Soil enables primary production and biodiversity	Physical anchorage for plants, nutrient cycling, habitat for billions of microorganisms, faunal habitat (earthworms, arthropods)	Soil fertility, biodiversity conservation, ecosystem resilience
Cultural	Soil has spiritual, aesthetic, recreational, and heritage value	Sacred soils, gardening as therapy, educational value, archaeological soil records, land-based identity	Mental health, cultural identity, traditional knowledge, ecotourism

Source: Authors' synthesis based on Adhikari and Hartemink (2016), the Food and Agriculture Organization of the United Nations [FAO] (2023), and Wall et al. (2012).

4.1. Soil ecosystem services

Soils provide multiple ecosystem services that are commonly grouped as regulating, provisioning, supporting, and cultural services (Adhikari & Hartemink, 2016). Regulating services include carbon storage, water

purification, flood regulation, and nutrient cycling. Because the global soil organic-carbon pool is much larger than the atmospheric carbon pool, relatively small proportional changes in soil carbon can influence atmospheric CO_2 concentrations (Lal, 2015). More than 95% of global food production depends directly or indirectly on soils, which also support fiber, biomass energy, raw materials, and microbial sources of pharmaceutical compounds (FAO, 2023; Wall et al., 2012). Supporting functions include root anchorage, nutrient supply, and habitat for immense biological diversity (Fierer, 2017). Cultural services encompass spiritual, educational, recreational, heritage, and identity-related values associated with soils and landscapes (Richter, 2007).

4.2. The two-way relationship between soil and climate change

The relationship between soil and climate change is bidirectional and involves multiple feedback loops (Davidson & Janssens, 2006). On one hand, climate change - through rising temperatures, altered precipitation patterns, and increased frequency of droughts and floods - directly affects soil formation processes, organic carbon dynamics, moisture regimes, and biodiversity. On the other hand, soil acts both as a source and as a sink of greenhouse gases. Agricultural soils emit N_2O, CO_2, and CH_4, but they also have a large potential to sequester carbon when managed sustainably (Paustian et al., 2016).

Practices such as intensive tillage, residue burning, poorly diversified cropping, and overgrazing can accelerate soil-organic-carbon losses (Montgomery, 2007). Cover crops, organic amendments, diversified rotations, agroforestry, and reduced soil disturbance can maintain or increase soil carbon under suitable conditions, but their effects vary with climate, soil type, baseline management, duration, and sampling depth (Lal, 2015; Paustian et al., 2016). Reduced tillage may redistribute carbon toward the surface without consistently increasing whole-profile stocks, and associated N_2O emissions can reduce the net climate benefit of some practices (Guenet et al., 2021). Soil-carbon sequestration should therefore be evaluated through complete greenhouse-gas accounting rather than as a universally positive outcome.

4.3. Soil and human health: the One Health perspective

The complex relationships between soil and health are increasingly examined through One Health and Soil Security perspectives, which connect human, animal, plant, ecosystem, and planetary health (Brevik et al., 2020;

Swan et al., 2024; Wall et al., 2012). Direct contact is one pathway: people encounter soil through agriculture, construction, mining, and recreation. Soil may contain pathogens such as *Clostridium tetani* and *Bacillus anthracis*, as well as pathogenic fungi or helminth eggs. Conversely, exposure to diverse environmental microbiota has been investigated as a contributor to immune regulation, although outcomes depend on the organisms, exposure route, dose, and host context (Rook, 2013). The remaining pathways operate through food, water, and air.

Food pathway: Soil properties influence the supply and bioavailability of essential nutrients to crops, but soil nutrient deficiency must not be equated directly with human clinical deficiency. An estimated 17.3% of the global population was at risk of inadequate dietary zinc intake in a major global assessment; this risk reflects interacting factors that include food systems, diets, crop composition, and soil conditions (Wessells & Brown, 2012). Selenium deficiency and toxicity are likewise geographically variable. Conversely, potentially toxic elements such as lead, cadmium, arsenic, and mercury may enter food chains through soil-crop pathways and create serious health risks (Alloway, 2013).

Water pathway: Healthy soils act as natural filters, absorbing rainwater, retaining pollutants, and recharging groundwater. Soil degradation and erosion weaken these functions, leading to water contamination and scarcity (Wall et al., 2012).

Air pathway: Wind erosion and dust storms, particularly in arid and semi-arid regions, mobilize large quantities of mineral particles and associated biological material. Long-range dust transport can disperse microorganisms and increase exposure to inhalable particulate matter, with documented or plausible respiratory-health implications (Griffin, 2007; Ravi et al., 2011). Specific disease burdens and cardiovascular effects should be evaluated with dedicated epidemiological evidence rather than inferred from transport studies alone.

Thus, the third paradigm shift in soil science integrates climate science, ecology, microbiology, and public health, emphasizing that soil management is not only an agricultural but also a climate and health imperative.

5. FOURTH PARADIGM: DIGITAL, BIOTECHNOLOGICAL, AND TRANSDISCIPLINARY SOIL SCIENCE

The fourth transition proposed here is emerging rather than complete. It is driven by digital technologies, new capacity to investigate and manipulate microbiomes, and recognition that soil problems are embedded in social, economic, legal, cultural, and political systems. The following directions illustrate its potential as well as its unresolved risks.

5.1. Digital soil science and the data revolution

Remote sensing, geographic information systems, proximal sensing, machine learning, and artificial intelligence are extending the foundations of digital soil mapping (Lagacherie, 2008; McBratney et al., 2003). These tools support spatial prediction, monitoring, uncertainty analysis, and decision support across soil properties and processes (Padarian et al., 2019; Zeng et al., 2025). Predictive accuracy, however, is not equivalent to process understanding. Methods such as LIME and SHAP can improve the local or global interpretation of model outputs, but they do not by themselves establish causality or eliminate bias in training data (Lundberg et al., 2020; Ribeiro et al., 2016). Explainable AI should therefore complement process knowledge, uncertainty reporting, independent validation, and transparent documentation. Table 4 summarizes the principal digital and biotechnological tools, opportunities, and limitations.

Table 4. Digital and biotechnological tools shaping the future of soil science

Tool / Technology	Primary application in soil science	Example	Key opportunity	Current challenge
Remote sensing (satellite, drone)	Mapping soil properties (moisture, organic carbon, texture) at large scales	Sentinel-2, Landsat, UAV-borne hyperspectral	High spatial/temporal coverage	Atmospheric interference, need for ground truthing
Artificial intelligence / machine learning	Predicting soil properties, classifying soil types, modelling soil processes	Random Forest, deep neural networks, LSTM	Handling big data, capturing non-linear relationships	Black-box nature, interpretability (need for XAI)
Internet of Things (IoT)	Real-time soil monitoring (moisture, temperature, electrical conductivity)	Wireless sensor networks, soil probes	Continuous data streams, precision agriculture	Sensor cost, energy supply, data transmission

Tool / Technology	Primary application in soil science	Example	Key opportunity	Current challenge
Metagenomics / omics	Characterizing soil microbiome composition and function	16S rRNA sequencing, metatranscriptomics	Culture-independent, high-resolution biodiversity assessment	Computational demands, functional interpretation
Synthetic biology	Research-stage design or modification of soil and plant-associated microbes	Designed microbial consortia; CRISPR-based modification of rhizobacteria	Hypothesis-driven manipulation of selected microbial functions	Ecological persistence, non-target effects, biosafety, regulation, and acceptance

Source: Authors' synthesis based on McBratney et al. (2003), Fierer (2017), Padarian et al. (2019), Lundberg et al. (2020), Singh and Ramakrishna (2021), and Zeng et al. (2025).

5.2. Manipulation of the soil microbiome and biotechnological advances

Omics approaches - including metagenomics, metatranscriptomics, proteomics, and metabolomics - have expanded the characterization of soil-microbiome composition and functional potential (Fierer, 2017). This knowledge supports efforts to manage microbial communities for plant growth, pathogen suppression, nutrient cycling, and pollutant degradation (Jansson & Hofmockel, 2020). Designed microbial consortia and CRISPR-based modification of plant-associated bacteria remain research-stage approaches rather than routine soil-management tools (Singh & Ramakrishna, 2021). Their prospective benefits must be evaluated alongside ecological persistence, horizontal gene transfer, non-target effects, biosafety, regulation, and public acceptance.

5.3. Soil and society: toward transdisciplinary soil research

Land-use and soil-management decisions reflect biophysical evidence as well as social, cultural, economic, legal, and political forces (Richter, 2007). Interdisciplinary work combines academic disciplines; transdisciplinary work goes further by involving non-academic actors in shared problem framing, knowledge co-production, implementation, and evaluation (Hopmans, 2020; Lang et al., 2012). Farmers, landholders, Indigenous and local communities, extension specialists, policy makers, and civil-society organizations may therefore participate as knowledge partners rather than only as recipients of

scientific recommendations. Terms such as human-soil relations, the social dimensions of soil, and soil justice can be useful analytical labels, but they should not be presented as a single, already standardized subdiscipline.

5.4. The digital divide and technology justice

Digital soil technologies may create or deepen inequalities when access to connectivity, sensors, software, capital, training, and technical support is uneven. Data ownership, privacy, commercial control, interoperability, and the capacity to contest automated recommendations are therefore substantive soil-governance issues, not merely technical details (Bronson, 2019; Klerkx et al., 2019). Low-cost and offline-capable tools, multilingual interfaces, open standards, participatory design, clear data-use agreements, and public infrastructure can reduce exclusion. Open data alone is insufficient if communities lack the resources or authority required to use it effectively.

6. CONCLUSION

Since its establishment as an independent discipline, soil science has undergone overlapping expansions in scope, method, and societal relevance (Amundson et al., 2015; Dokuchaev, 1883; Jenny, 1941; Lehmann et al., 2020). The first transition established soil as an independent natural body and provided foundations for pedogenesis and classification. The second combined recognition of soil as a living system with environmental concern for pollution, conservation, and remediation. The third positioned soil as a provider of ecosystem services, a component of climate feedbacks, a reservoir of biodiversity, and a determinant of human and environmental health (Lal, 2015; Swan et al., 2024; Wall et al., 2012).

The proposed fourth transition integrates three tendencies. First, soil management is increasingly examined within social, economic, legal, and cultural systems (Montgomery, 2007; Richter, 2007). Second, digital tools expand mapping, monitoring, and prediction while raising questions about uncertainty, interpretability, privacy, ownership, and unequal access (Bronson, 2019; Padarian et al., 2019). Third, transdisciplinary research connects academic disciplines with non-academic knowledge and decision making (Hopmans, 2020; Lang et al., 2012). In this chapter, One Soil is proposed explicitly as a heuristic label - not as an already established formal framework - for considering soil's physical, chemical, biological, ecological, economic,

social, cultural, and ethical dimensions together. Its value should ultimately be judged by whether it improves analysis, participation, and soil-governance outcomes.

Several priorities follow from this synthesis. Research should combine predictive performance with uncertainty analysis, process knowledge, explainability, and independent validation. Soil-science education can retain strong training in pedology, chemistry, physics, and biology while adding competence in ethics, governance, law, economics, history, and participatory methods. Policy should support interoperable data, transparent data-use rules, affordable monitoring infrastructure, and meaningful participation by smallholder farmers and other affected communities. These measures should be evaluated in specific social and environmental contexts rather than presented as universally transferable technical solutions.

The future of soil science therefore depends on linking rigorous biophysical understanding with responsible technological innovation and socially robust decision making. Soil classification, process research, and measurement remain indispensable; the emerging task is to connect them more effectively with health, climate, governance, and lived experience. A transdisciplinary orientation can strengthen this connection when it is based on explicit methods, transparent evidence, shared problem framing, and accountable participation.

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CHAPTER 15

THE ROLE OF SOUND FREQUENCIES IN POSTHARVEST PHYSIOLOGY: SPECIES-SPECIFIC PROTOCOLS, MECHANISMS, AND OPPORTUNITIES FOR TRANSLATION TO COMMERCIAL STORAGE

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INTRODUCTION

Postharvest deterioration is governed by interacting physiological, biochemical, physical and microbial processes, and interventions that slow one process can create unintended effects on another quality attribute. The practical objective is therefore not simply to delay visible ripening, but to preserve marketable quality, safety and sensory acceptability across storage, transport and retail while maintaining an economically defensible process (Jiang et al., 2020).

Interest in sound-based interventions has increased because mechanical energy can be applied without leaving a conventional chemical residue on the commodity. The term ‘sound treatment’, however, has often been used imprecisely for airborne audible vibration, immersion ultrasound, thermosonication, acoustic cleaning, and non-destructive acoustic measurements, although these systems operate through different coupling media and different energy-transfer mechanisms (Hassanien et al., 2014; Taniwaki & Sakurai, 2010; Jiang et al., 2020; Zhou et al., 2022).

Airborne audible sound is commonly delivered by loudspeakers in the approximate human-audible range and reaches produce through pressure fluctuations in air and through vibration of surrounding structures. Power ultrasound is normally delivered above 20 kHz through water or another liquid and can generate cavitation, microstreaming and shear, while acoustic sensing applies low-energy excitation and analyses the product response rather than attempting to alter physiology (Taniwaki & Sakurai, 2010; Mishra et al., 2016; Jiang et al., 2020).

The distinction is more than semantic. Two systems with the same nominal frequency can deliver different doses because sound pressure, particle velocity, source distance, waveform, exposure time, chamber or tank geometry, load distribution, temperature and coupling conditions determine the physical field experienced by the tissue (Hassanien et al., 2014; Jiang et al., 2020; Zhou et al., 2022).

The published evidence is uneven. Airborne audible-sound studies are concentrated in a small number of tomato experiments, whereas power-ultrasound studies span more commodities but frequently involve different bath designs or combined treatments. Accordingly, only claims whose intervention,

medium, protocol and outcome could be verified from accessible primary or authoritative sources are retained in this chapter.

The aims of this chapter are to define the relevant acoustic modalities, explain the physical and physiological basis of each modality, critically evaluate direct harvested-produce evidence, develop source-aligned candidate protocols for the commodities that have been studied, identify methodological weaknesses that limit reproducibility, and propose a stage-gated pathway for commercial storage and packing-house validation.

1. SCOPE, SOURCE VERIFICATION, AND EVIDENCE GRADING

1.1. Review design and source-verification rule

This chapter uses a critical evidence-synthesis approach rather than presenting a PRISMA systematic review. Sources were retained only when their bibliographic identity and the methods and results relevant to the stated claim could be verified.

A study was eligible for quantitative protocol tables only when the full text could be accessed and the commodity, maturity, acoustic medium, frequency, level or power, duration, temperature and storage context could be checked. Reviews were used to define physics, terminology and broader limitations, whereas numerical treatment prescriptions were taken from primary studies whenever possible (Jiang et al., 2020; Esua et al., 2020; Zhou et al., 2022).

Evidence was graded by inferential distance. ‘Direct postharvest evidence’ required an intervention applied after harvest to intact or minimally processed produce; ‘supporting plant-mechanobiology evidence’ included intact plants or model species used to interpret signalling; ‘process-engineering evidence’ addressed cavitation, acoustic field, cleaning or scale-up; and ‘commercial inference’ identified a requirement that has not yet been demonstrated as an efficacy claim (Table 1).

A claim-to-source matrix was used to maintain traceability. Each quantitative statement was linked to the corresponding primary study, and mechanistic statements were classified according to whether they were measured directly in harvested produce or inferred from another biological system.

Table 1. Evidence classes used to interpret the postharvest acoustics literature

Evidence class	Minimum requirement	Permitted conclusion	Prohibited overinterpretation
A. Direct postharvest intervention	Harvested commodity; defined acoustic treatment; concurrent control; postharvest outcome measured.	The reported treatment affected the measured outcome under the stated commodity and storage conditions.	Universal species response, commercial effectiveness, or an unmeasured molecular receptor.
B. Mechanistic postharvest evidence	Harvested commodity plus gene, protein, metabolite, enzyme or hormone measurements.	The measured pathway is associated with the treatment response.	Causality of an upstream receptor without inhibition, genetics or direct imaging.
C. Supporting plant mechanobiology	Intact plant, seedling, callus or model species exposed to sound or mechanical stimulation.	The pathway is biologically plausible and can guide a hypothesis in harvested tissue.	Direct confirmation in harvested fruit or transfer of the reported dose.
D. Process-engineering evidence	Measured or modelled acoustic/cavitation field, tank/room geometry, cleaning or process variables.	The process variable can influence delivered dose, uniformity, injury or scale-up.	Biological efficacy in a commodity without a commodity trial.
E. Commercial inference	Requirement derived from food-process validation, safety, economics or quality management.	A criterion that must be tested before adoption.	A claim that commercial adoption is already technically or economically proven.

Source: Author's evidence-grading framework developed from the methodological boundaries in Kim et al. (2015, 2018), Jiang et al. (2020), Esua et al. (2020), and Zhou et al. (2022).

1.2. Terminological boundaries

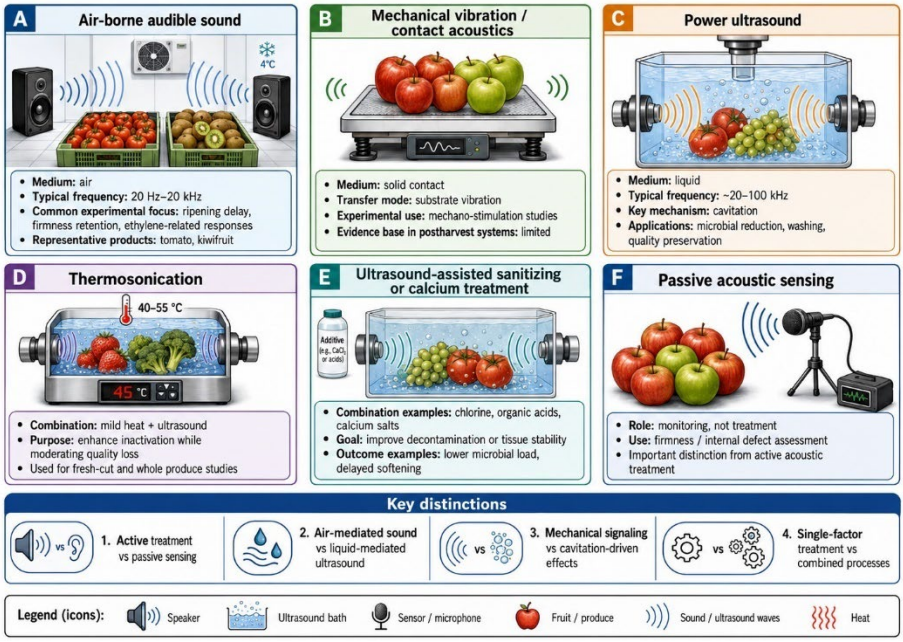
The lower boundary of human hearing is commonly placed near 20 Hz and the upper boundary near 20 kHz, although practical hearing limits vary with age and sound pressure. In this chapter, 'airborne audible sound' refers to loudspeaker-generated pressure waves below 20 kHz, while 'power ultrasound' refers to processing systems above 20 kHz that are usually coupled through a liquid (Hassanien et al., 2014; Jiang et al., 2020).

The expression 'low-frequency sound' is potentially confusing because a frequency of 1 kHz is low relative to ultrasound but is not low relative to much of the audible spectrum. The chapter therefore reports the numerical frequency and the coupling medium rather than relying on the adjective 'low-frequency' alone.

Acoustic sensing is included because it is relevant to a future feedback-controlled storage system, but it is not counted as a preservation treatment. Resonance and impact methods can estimate firmness or maturity by relating vibration response to mass, shape and mechanical properties, whereas they do

not by themselves demonstrate a physiological treatment effect (Taniwaki & Sakurai, 2010).

The acoustic modalities that must be distinguished when evaluating postharvest applications are presented in Figure 1, and the physical descriptors required to define an acoustic treatment dose are provided in Table 2.



Source: Author's original synthesis based on Hassanien et al. (2014), Taniwaki and Sakurai (2010), Mishra et al. (2016), Jiang et al. (2020), and Zhou et al. (2022).
Figure 1. Acoustic modalities that must be separated when evaluating postharvest applications.

Table 2. Physical descriptors required to define an acoustic treatment dose

Descriptor	Airborne audible sound	Power ultrasound	Why it matters
Frequency	Hz or kHz at the product location.	kHz; single, dual or multifrequency mode.	Frequency influences wavelength, resonance and cavitation behaviour but does not define dose by itself.
Level or power	Sound-pressure level in dB with reference and weighting; particle velocity when available.	Nominal electrical power, delivered/calorimetric power, W L^{-1} or W cm^{-2}.	Equipment settings may not equal energy delivered to the product.
Waveform and duty cycle	Sine, swept, noise; continuous or intermittent.	Continuous or pulsed; amplitude and pulse schedule.	Peak and time-averaged exposure can differ substantially.
Geometry	Source distance, speaker orientation, room dimensions, load and package arrangement.	Tank dimensions, transducer position, water depth, product load and rack/basket.	Standing waves, shadow zones and load-dependent attenuation alter treatment uniformity.
Medium state	Air temperature, humidity, background noise and structural vibration.	Water temperature, dissolved gas, pH, turbidity, sanitizer and recirculation.	Medium properties affect propagation, cavitation and

			confounding thermal/chemical effects.
Exposure time	Single duration and any repeated schedule.	Residence time and number of treatment passes.	Biological benefit and tissue injury are often dose-window responses.
Spatial verification	Microphone/SPL map around product positions.	Hydrophone, foil, sonochemiluminescence or calorimetric mapping.	A single equipment label cannot demonstrate uniform dose.

Source: Compiled from Hassanien et al. (2014), Jiang et al. (2020), Esua et al. (2020), and Zhou et al. (2022).

2. PHYSICAL BASIS OF SOUND–TISSUE INTERACTIONS

2.1. Airborne audible pressure waves

A loudspeaker converts an electrical signal into alternating pressure and particle motion in air. The pressure field reaching fruit depends on source output, distance, reflections and the acoustical impedance of boundaries, and a loaded cold room can therefore contain pressure maxima, minima and shadow zones even when the signal generator produces a stable frequency (Hassanien et al., 2014; Mishra et al., 2016).

At the commodity surface, acoustic pressure may induce very small displacements of the cuticle, epidermis, internal air spaces and support structure. Whether the biologically relevant variable is airborne pressure, structural vibration, tissue acceleration, a resonance phenomenon or a combination of these variables has not been resolved for harvested fruit (Mishra et al., 2016; Jung et al., 2018).

The tomato studies reported loudspeaker frequency, duration and sound-pressure level, but they did not provide a three-dimensional dose map within the chamber or a complete mechanical transfer function from speaker to fruit tissue. Their findings establish a repeatable treatment concept within one research programme, while the delivered tissue-level mechanical dose remains incompletely characterized (Kim et al., 2015, 2018; Jeong et al., 2023).

2.2. Liquid-coupled power ultrasound and cavitation

Power ultrasound is transmitted efficiently through a liquid, where alternating compression and rarefaction can produce the growth and collapse of cavitation bubbles. Bubble dynamics can generate microstreaming, jets, shear, localized pressure and temperature excursions, radical chemistry and enhanced transport at the produce surface (Jiang et al., 2020; Esua et al., 2020; Zhou et al., 2022).

These processes can detach soil and microorganisms, disrupt microbial structures, modify enzyme activity, and act as a physical elicitor of tissue

responses. The same processes can also damage the cuticle, soften tissue, accelerate leakage or cause nutrient loss when intensity or duration exceeds a commodity-specific tolerance window (Jiang et al., 2020; Zhou et al., 2022; Muzaffar et al., 2016).

Ultrasound combined with heat, calcium or sanitizer cannot be interpreted as ultrasound alone. A thermosonication experiment includes a thermal contribution, a calcium-assisted experiment includes cell-wall cross-linking and ionic effects, and a chlorine-assisted wash includes chemical lethality; factorial controls are required to estimate the contribution and interaction of each component (Pinheiro et al., 2019; Supapvanich et al., 2022; Wang et al., 2022).

2.3. Low-intensity acoustic sensing

Acoustic vibration methods can characterize the mechanical state of horticultural products by measuring resonant frequency, damping, transmitted waves or impact response. Because firmness and internal structure influence dynamic mechanical behaviour, acoustic indices can be calibrated against destructive reference measurements for maturity or texture assessment (Taniwaki & Sakurai, 2010).

A sensing system could eventually support adaptive treatment by identifying lots that require different exposure or by monitoring firmness change during storage. Such a closed-loop concept is an engineering opportunity, not an established commercial outcome of the current treatment literature (Taniwaki & Sakurai, 2010).

3. POSTHARVEST PHYSIOLOGICAL TARGETS AND MECHANISTIC INTERPRETATION

3.1. Mechanical perception: a plausible but incompletely tested entry point

Plant cells possess multiple systems that convert membrane tension, wall deformation and mechanical strain into electrical and biochemical signals. Mechanosensitive channels can respond rapidly to membrane tension, and mechanical stimulation is frequently followed by transient cytosolic calcium elevation and changes in reactive oxygen species; however, the identity and contribution of specific channels depend on tissue and stimulus context (Peyronnet et al., 2014).

Sound-vibration studies in Arabidopsis have shown changes in transcripts, proteins, hormones and stress-related pathways, and several sound-responsive genes overlap with genes induced by touch. These observations support a mechanosensory interpretation, but they do not demonstrate that the same receptor initiates the delayed-ripening phenotype in harvested tomato (Ghosh et al., 2016, 2017; Jung et al., 2018).

The mechanosensitive-channel–calcium–ROS–kinase sequence should therefore be presented as a testable upstream model. Direct confirmation would require tissue-level vibration measurement, real-time calcium imaging, membrane-potential or ion-flux measurements, pharmacological or genetic perturbation, and demonstration that blocking the candidate pathway abolishes the postharvest phenotype (Peyronnet et al., 2014; Mishra et al., 2016).

3.2. Ethylene biosynthesis and climacteric ripening

The strongest direct mechanistic evidence concerns the ethylene network in mature-green tomato. A 1 kHz, 6 h treatment was associated with lower ethylene production, lower respiration, delayed surface-colour development and slower firmness loss, together with altered expression of ACS2, ACS4, ACO1, E4, E8 and ripening-regulatory transcription factors including RIN, TAGL1, HB-1, NOR and CNR (Kim et al., 2015).

RNA sequencing subsequently showed that ethylene- and cytokinin-related genes and cell-wall-modification processes were altered after treatment, while flavonoid, phenylpropanoid and glucan-related pathways were represented among the responses. The study also identified sound-associated non-coding RNAs and candidate microRNA–target relationships, expanding the response beyond a small set of preselected genes (Kim et al., 2018).

The available evidence supports the statement that the tested sound treatment modulated the ethylene/ripening network in the studied tomato system. It does not support the broader statement that audible sound suppresses ethylene in every climacteric commodity or that 1 kHz is optimal for other cultivars, maturity stages or storage temperatures (Kim et al., 2015, 2018).

3.3. Epigenetic regulation

A later study reported that the sound treatment altered histone-modification patterns at ripening-related loci. Activating histone marks were reduced and repressive marks increased at RIN, NOR and TAGL1 and at

ethylene-biosynthesis-related genes, while the expression of genes encoding histone-modifying enzymes was also affected (Kim et al., 2023).

The epigenetic findings provide a plausible explanation for persistence of transcriptional effects after a finite exposure, but they remain part of the same tomato research lineage rather than an independent replication. The duration, reversibility and cultivar dependence of the chromatin state require further study before an epigenetic memory can be used as a process-control claim (Kim et al., 2023).

3.4. Primary metabolism and respiratory progression

Metabolomic profiling after 1 kHz treatment identified differences in metabolites measured at 7, 10 and 14 d. Treated fruit had lower adenosine, tryptophan, phytosphingosine, 4-aminobutanoic acid and aspartic acid and higher malic acid, citric acid and sucrose among the metabolites highlighted by the authors, which was interpreted as broadly consistent with altered ripening progression (Jeong et al., 2023).

Metabolite associations do not establish which change is causal, and the experiment used three biological replicates and a single cultivar under room-temperature storage. The results are valuable as multi-omics coherence with the earlier physiology and transcriptomic data, but they should not be converted into a universal metabolic signature of acoustic treatment (Jeong et al., 2023).

3.5. Cell-wall disassembly and firmness

Firmness loss during fruit ripening reflects coordinated changes in pectin, hemicellulose, cellulose, middle-lamella adhesion, turgor and water status. In the tomato audible-sound work, delayed firmness loss accompanied delayed ripening and cell-wall-related transcriptomic responses, although direct activities of individual cell-wall enzymes were not comprehensively established in the original experiment (Kim et al., 2015, 2018).

The strawberry ultrasound study provides more direct cell-wall evidence. A 28 kHz, 0.15 W cm^{-2}, 3 min immersion treatment reduced the rate of firmness loss and soluble-pectin increase and was associated with lower pectin esterase and polygalacturonase activity during cold storage, together with transcriptomic changes in ROS and hormone-related pathways (Zhang et al., 2022).

The blueberry ultrasound study linked texture retention with higher lignin content and altered expression in phenylpropanoid, flavonoid and plant–

pathogen interaction pathways. These data support a commodity-specific elicitation response, but the reported 25 kHz, 2 min condition should not be transferred without confirming the actual delivered power and the cultivar and storage context (Hou et al., 2024).

3.6. Oxidative balance, phenylpropanoid metabolism and defence

Low levels of reactive oxygen species can function as signals, whereas excessive accumulation contributes to membrane oxidation and senescence. Ultrasound can simultaneously create physicochemical radicals in the liquid and stimulate tissue antioxidant responses, making it essential to distinguish measured tissue ROS and enzyme activity from cavitation chemistry in the surrounding medium (Jiang et al., 2020; Zhou et al., 2022).

In blueberry, ultrasound-treated fruit showed lower superoxide and hydrogen peroxide and higher activities of antioxidant enzymes, while transcriptomic results implicated phenylpropanoid and lignin-related responses. In strawberry, the authors proposed that altered ROS and jasmonate signalling delayed ethylene-related responses and pectin degradation; both models are supported by physiological and transcriptomic associations rather than receptor-level causality (Zhang et al., 2022; Hou et al., 2024).

Sound-vibration experiments in Arabidopsis provide indirect support for defence priming because 1 kHz pretreatment increased resistance to Botrytis cinerea and altered salicylic-acid-responsive genes and proteins. These plant-pathology results cannot be cited as direct evidence that an airborne sound treatment suppresses postharvest decay in fruit, because the biological system, exposure schedule and infection context differ (Choi et al., 2017; Ghosh et al., 2019).

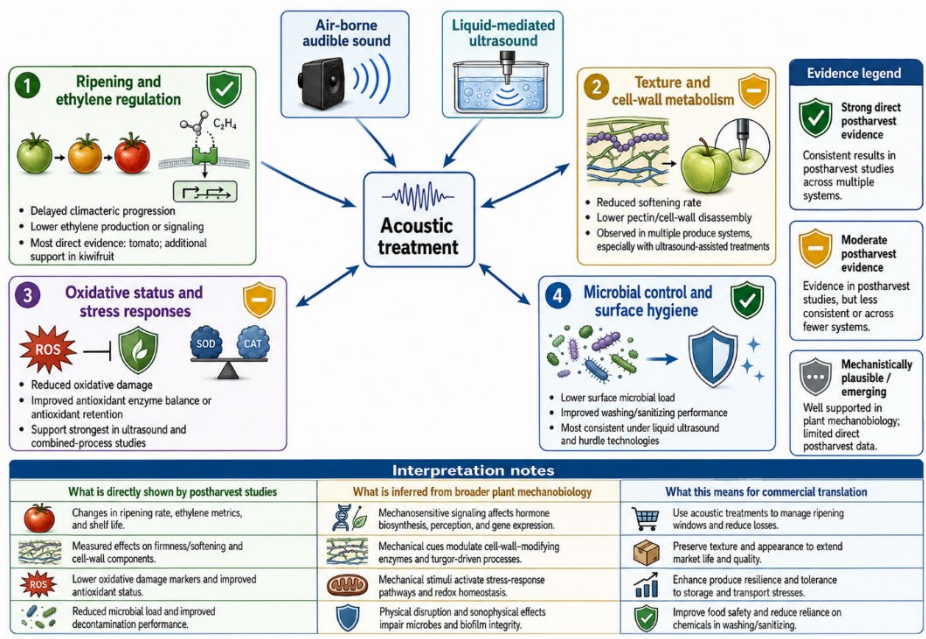
3.7. Microbial inactivation versus host-mediated resistance

Direct microbial inactivation is most relevant to liquid-coupled ultrasound, where cavitation and shear can detach or injure microorganisms and can enhance a sanitizer. Host-mediated resistance refers to changes in the produce tissue that alter susceptibility, and the two processes must be separated experimentally because they operate on different targets (Esua et al., 2020; Zhou et al., 2022).

The winter-jujube study illustrates this distinction. Ultrasound at 28 kHz alone did not prevent pathogen cross-contamination under the tested wash conditions, whereas the combination of ultrasound and 10 ppm free chlorine

reduced the tested cross-contamination incidence to nondetectable levels and maintained measured quality during seven days at 4 °C (Wang et al., 2022).

The study therefore supports a hurdle-process conclusion, not the conclusion that ultrasound alone is an adequate disinfection step. Commercial wash-water claims require validation against the relevant pathogen or surrogate, water reuse, organic load, detection limit and process critical limits (Wang et al., 2022; Esua et al., 2020).



Source: Author's original synthesis based on Peyronnet et al. (2014), Kim et al. (2015, 2018, 2023), Ghosh et al. (2016, 2017, 2019), Choi et al. (2017), Jiang et al. (2020), Zhang et al. (2022), Jeong et al. (2023), and Hou et al. (2024).

Figure 2. Evidence-graded mechanistic framework for airborne sound and power ultrasound in postharvest systems.

Evidence-graded mechanistic framework for airborne sound and power ultrasound in postharvest systems are presented in Figure 2, and Mechanistic endpoints, direct evidence status, and interpretation limits are provided in Table 3.

Table 3. Mechanistic endpoints, direct evidence status, and interpretation limits

Endpoint	Direct harvested-produce evidence	Supporting evidence	Defensible interpretation
Ethylene production and respiration	Lower in 1 kHz-treated mature-green tomato; delayed climacteric progression.	Transcriptomic and epigenetic changes in ethylene/ripening genes.	Strong within the reported tomato system; unvalidated across species and commercial storage.
Ripening transcription factors	RIN, TAGL1, HB-1, NOR and CNR expression affected in tomato.	Histone-mark changes at RIN, NOR and TAGL1 loci.	The treatment is associated with transcriptional and chromatin regulation of tomato ripening.
Cell-wall metabolism	Firmness retained in tomato; PE/PG and soluble pectin affected in strawberry ultrasound study.	Cell-wall-related transcriptomic pathways in tomato and strawberry.	Texture response is supported, but the responsible pathway is modality- and commodity-specific.
Primary metabolites	LC–MS/GC–MS differences in treated tomato at 7–14 d.	Consistency with delayed colour/ripening progression.	Metabolomic association, not proof of causal metabolites.
ROS and antioxidants	Measured in strawberry and blueberry ultrasound studies.	Arabidopsis sound-vibration transcriptomic/proteomic responses.	Supports an elicitation model for ultrasound; airborne harvested-fruit confirmation remains limited.
Phenylpropanoid/lignin	Lignin and pathway expression affected in blueberry ultrasound study.	Sound-responsive defence pathways in Arabidopsis.	A plausible texture/defence mechanism in blueberry under the tested protocol.
Mechanosensitive channels and Ca^{2+}	No direct receptor or calcium-imaging demonstration in harvested fruit in the core studies.	General plant mechanobiology and sound/touch response studies.	Hypothesis that must be labelled as extrapolation.
Microbial control	Direct counts in cherry; pathogen cross-contamination study in winter jujube combination wash.	Reviews of cavitation and hurdle technologies.	Process-specific; ultrasound alone cannot be assumed to provide adequate lethality.

Source: Compiled from the primary and review studies cited in each row; interpretation boundaries follow the evidence classes in Table 1.

4. DIRECT POSTHARVEST EVIDENCE FOR AIRBORNE AUDIBLE SOUND

4.1. The 2015 tomato physiology experiment

Kim et al. (2015) harvested mature-green tomato fruit and screened 0, 250, 500, 800, 1000 and 1500 Hz for 6 h before room-temperature storage. The strongest delay was observed at 1 kHz, which was selected for detailed monitoring over the following fourteen days at 23 ± 1 °C (Kim et al., 2015).

Seven days after treatment, more than 85% of the treated fruit remained green compared with fewer than 50% of the untreated fruit, although most fruit in both groups reached the red stage by day 14. Ethylene production and respiration were lower, the green-to-red colour transition was delayed, and flesh firmness declined more gradually in treated fruit (Kim et al., 2015).

The experiment used three biological replicate experiments for phenotypic analyses and reported 32 fruit for ethylene measurements across

time. The paper established a postharvest proof of concept, but it did not include a cold-storage treatment, another cultivar, a loaded commercial chamber or an independent laboratory (Kim et al., 2015).

4.2. Coding and non-coding RNA response

Kim et al. (2018) exposed mature-green 'Dotaerang' tomato to 1 kHz for 6 h at 80 dB in a soundproof chamber and stored the fruit at 23 ± 1 °C and 60 ± 2% relative humidity. Samples were collected at 6 h and 2, 5 and 7 d for RNA sequencing and validation (Kim et al., 2018).

The study identified time-dependent changes in hormone biosynthesis and signalling, cell-wall modification, flavonoid, phenylpropanoid and glucan-related processes and identified candidate sound-associated microRNAs and targets. The authors acknowledged limited sample numbers and used fold-difference screening for parts of the transcriptomic selection, which increases the importance of independent validation of specific biomarkers (Kim et al., 2018).

4.3. Metabolomic coherence

Jeong et al. (2023) used 1 kHz for 6 h at 100 dB and analysed fruit at 7, 10 and 14 d using LC–MS and GC–MS. Three biological replicates were selected at each comparison, and the authors highlighted 33 major metabolites using variable-importance and probability criteria (Jeong et al., 2023).

The morphology and multivariate metabolite patterns were consistent with delayed ripening, and selected organic acids and sucrose were higher while selected amino-acid and lipid-related metabolites were lower in treated fruit. Because the fruit and treatment came from the same general research system as the earlier studies, the work strengthens internal coherence but not external independence (Jeong et al., 2023).

4.4. Histone modification and persistence of response

Kim et al. (2023) examined histone marks and expression of histone-modifying enzymes after the specific sound treatment. The study reported reduced activating and increased repressive histone modifications at major ripening regulators and ethylene-biosynthesis-related loci, providing a chromatin-level model for transcriptional suppression (Kim et al., 2023).

The finding is mechanistically important because a finite physical exposure could influence later ripening only if the induced signal changes downstream regulatory states. The study does not yet show how long the chromatin changes persist, whether they occur at cold-storage temperature, or whether they are reproduced in other tomato genotypes (Kim et al., 2023).

4.5. Preharvest intact-plant evidence and its boundary

Altuntas and Ozkurt (2019) exposed ten-week-old tomato plants sequentially to 600, 1240 and 1600 Hz at 90 dB over three weeks and measured fruit composition at harvest. Lycopene, vitamin C, sugars, acids and phenolics increased with the sequence of treatments, but the intervention was applied to intact growing plants rather than harvested fruit (Altuntas & Ozkurt, 2019).

This study demonstrates that plant developmental history can alter later fruit quality and confirms that sound responses are not confined to detached tissues. It cannot validate a postharvest storage protocol because the exposure timing, developmental state and causal pathway differ from treatment of harvested mature-green fruit (Altuntas & Ozkurt, 2019), (Table 4).

Table 4. Full-text-verified airborne sound studies relevant to tomato fruit.

Study	Biological material	Treatment and storage	Measured outcomes	Evidence and limitation
Kim et al. (2015)	Harvested mature-green tomato; detailed work at 1 kHz after initial frequency screen.	250, 500, 800, 1000 and 1500 Hz screened for 6 h; 1 kHz selected; 23 ± 1 °C for 14 d.	Ripening stage, ethylene, respiration, colour, firmness, qRT-PCR of ethylene and ripening genes.	Direct postharvest proof of concept; single research programme and room-temperature storage.
Kim et al. (2018)	Harvested mature-green 'Dotaerang' tomato.	1 kHz, 80 dB, 6 h; 23 ± 1 °C, 60 ± 2% RH; samples 6 h to 7 d.	RNA-seq, qRT-PCR, coding and non-coding RNAs, hormone and cell-wall pathways.	Mechanistic postharvest evidence; limited replication noted by authors.
Jeong et al. (2023)	Harvested mature-green tomato.	1 kHz, 100 dB, 6 h; 23 ± 1 °C; samples 7, 10 and 14 d.	LC–MS and GC–MS metabolomics; morphology; three biological replicates.	Metabolomic coherence; not an independent cultivar/laboratory replication.
Kim et al. (2023)	Harvested tomato within the established 1 kHz research system.	Specific sound treatment; analysis of ripening-related chromatin regulation.	Histone marks and histone-modifying enzyme expression at ripening and ethylene loci.	Epigenetic mechanism; persistence and external validity require validation.
Altuntas & Ozkurt (2019)	Ten-week-old intact tomato plants; fruit analysed at harvest.	600, 1240 and 1600 Hz applied sequentially by week at 90 dB.	Lycopene, vitamin C, sugar, acid, phenolics, pH and soluble solids.	Preharvest supporting evidence; not a harvested-fruit treatment.

Source: Direct extraction from the full texts of the cited studies. No frequency or percentage from an unverified commodity study was retained.

4.6. What the tomato programme does and does not establish

Across physiology, targeted gene expression, whole-transcriptome analysis, metabolomics and histone modification, the tomato studies form a coherent multi-level evidence chain. The coherence makes the 1 kHz, 6 h treatment a credible candidate protocol for replication in mature-green tomato, rather than a speculative concept (Kim et al., 2015, 2018, 2023; Jeong et al., 2023).

The studies do not establish a universal optimum frequency, because frequency screening was limited, treatment-level metrology was incomplete, and cultivar, maturity, temperature, package and atmosphere interactions were not mapped. The absence of independent multi-laboratory replication is the main reason the evidence should be described as proof of concept rather than an established commercial technology.

A rigorous replication should reproduce the original 'Dotaerang' condition, include a calibrated sham chamber, report spatial sound-pressure and vibration maps, predefine ethylene or colour as the primary endpoint, and add a second cultivar and cold-storage arm. Only after the reference phenotype is replicated should a broader response-surface experiment vary frequency, level, duration and duty cycle.

5. SPECIES-SPECIFIC POWER-ULTRASOUND PROTOCOLS

5.1. Strawberry: texture, pectin metabolism and aroma

Zhang et al. (2022) treated 'Benihoppe' strawberry by complete immersion in deionized water at 28 kHz and 0.15 W cm^{-2} for 3 min while maintaining the bath at 15 ± 1 °C. Control fruit were immersed for the same duration without ultrasound, and fruit were stored at 5 ± 1 °C and 75–85% relative humidity for up to 24 d (Zhang et al., 2022).

After 24 d, the authors reported a 41.70% lower firmness-reduction rate and a 63.12% lower rate of soluble-pectin increase relative to the control, while pectin esterase and polygalacturonase activities were lower at later storage points. Transcriptomic analysis identified 1,905 differentially expressed genes and implicated ROS, auxin, ethylene, jasmonate and transcription-factor responses (Zhang et al., 2022).

Li et al. (2024) used the same 28 kHz, 0.15 W cm^{-2}, 3 min treatment and storage at 5 °C to examine volatile organic compounds. The treatment altered the time course of esters, terpenes and other aroma compounds and was associated with expression of genes including FaNES and FaAAT, indicating that texture preservation should be evaluated together with flavour rather than inferred from firmness alone (Li et al., 2024).

The strawberry evidence is relatively strong for a candidate laboratory protocol because the water control, treatment temperature and several physiological and molecular outcomes were reported. External validity remains limited to the studied cultivar, bath, package and storage regime, and decay/sensory performance should be independently replicated before scale-up (Zhang et al., 2022; Li et al., 2024).

5.2. Blueberry: firmness, lignification and redox response

Hou et al. (2024) applied a 25 kHz ultrasound wash for 2 min using a reported power value of 400 W and evaluated blueberry texture, oxidative indicators and transcriptomic responses. Treated fruit had higher firmness and lignin, lower superoxide and hydrogen peroxide, and higher antioxidant-enzyme activity than controls under the reported conditions (Hou et al., 2024).

The transcriptomic analysis identified 163 differentially expressed genes and enriched phenylpropanoid, flavonoid and plant–pathogen interaction pathways, with lignin-related genes correlated with firmness. The protocol is a promising elicitation treatment, although the paper's equipment-level power description should be converted to delivered power density and spatial cavitation measurements before inter-laboratory transfer (Hou et al., 2024).

5.3. Sweet cherry: a clear duration–benefit–injury window

Muzaffar et al. (2016) treated sweet cherry at 33 kHz and 60 W for 10, 20, 30, 40 or 60 min and stored the fruit at 4 °C for 15 d. Treatments of approximately 20–40 min produced the best overall balance of colour, antioxidant properties and microbial load, whereas firmness declined significantly when exposure exceeded 20 min and 60 min produced detrimental effects on several attributes (Muzaffar et al., 2016).

The study is important because it demonstrates that a longer acoustic treatment is not automatically better. The reported optimum is specific to the

bath, load and cultivar, but the shape of the response supports routine inclusion of an injury boundary in ultrasound optimisation (Muzaffar et al., 2016).

5.4. Mature-green tomato: thermosonication rather than ultrasound alone

Pinheiro et al. (2019) optimized thermosonication of whole mature-green 'Zinac' tomato using 45 kHz ultrasound at 80% equipment power while varying temperature from 32 to 48 °C, treatment time from 13 to 47 min, and storage at 10 °C from 1 to 15 d. Response-surface analysis selected 32 °C/13 min, 35 °C/20 min and 40 °C/30 min for validation, with 40 °C/30 min providing the longest storage with high overall quality (Pinheiro et al., 2019).

The result cannot be attributed to ultrasound alone because temperature and acoustic treatment were intentionally integrated. A future factorial design should include water-only controls at every selected temperature and time, an ultrasound-only condition at matched temperature, and a calorimetric energy balance to quantify the interaction (Pinheiro et al., 2019).

5.5. Pink guava: calcium-assisted ultrasound

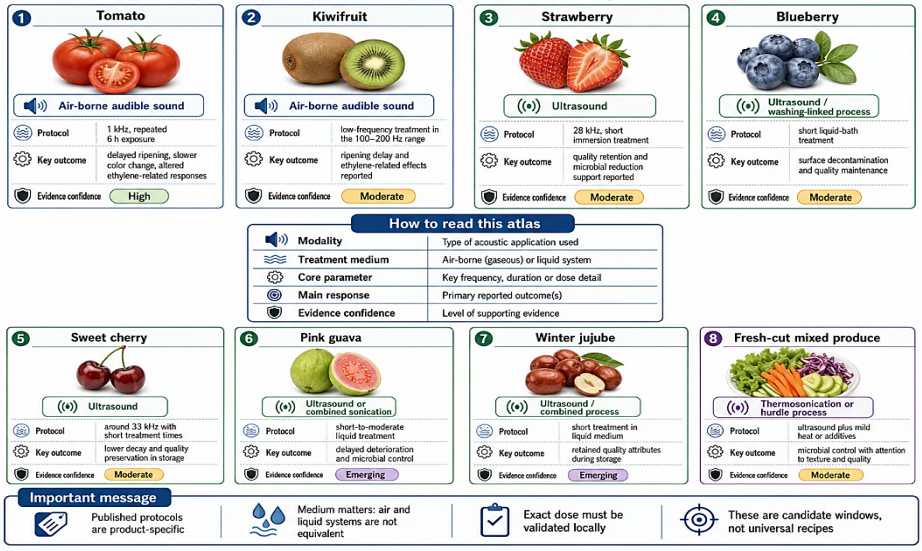
Supapvanich et al. (2022) combined 40 kHz, 150 W ultrasonication with 0.5% calcium gluconate and tested 2, 4, 6 and 8 min on pink guava stored at 27 ± 1 °C for 6 d. Four minutes produced the best reported balance for peel colour, black-spot symptoms, firmness, membrane integrity, ascorbic acid, phenolics and antioxidant activity (Supapvanich et al., 2022).

Calcium can stabilize cell-wall structure and membranes, so the result supports a combined process rather than a pure acoustic effect. Separate calcium-only and ultrasound-only controls are necessary when translating the protocol or attributing the mechanism (Supapvanich et al., 2022).

5.6. Winter jujube: cross-contamination requires a sanitizer hurdle

Wang et al. (2022) evaluated 28 kHz ultrasound and 10 ppm free chlorine, separately and in combination, for whole ready-to-eat winter jujube inoculated with Escherichia coli O157:H7, non-O157 E. coli and Salmonella Typhimurium. Ultrasound alone did not eliminate cross-contamination, while the combination reduced cross-contamination to below detection in the experimental system and maintained measured quality during 0–7 d at 4 °C (Wang et al., 2022).

This is a strong example of why a preservation chapter must not equate lower total counts with a validated pathogen-control step. The effective unit operation was the ultrasound–chlorine combination, and the critical process variables include sanitizer residual, organic load, water reuse and pathogen detection limit (Wang et al., 2022).



Source: Author's original synthesis from Kim et al. (2015, 2018, 2023), Muzaffar et al. (2016), Pinheiro et al. (2019), Wang et al. (2022), Zhang et al. (2022), Supapvanich et al. (2022), Jeong et al. (2023), Hou et al. (2024), and Li et al. (2024).

Figure 3. Full-text-verified species-specific candidate protocols for airborne sound and immersion ultrasound.

Full-text-verified species-specific candidate protocols for airborne sound and immersion ultrasound are presented in Figure 3, and Full-text-verified species-specific ultrasound protocols and transfer limits are provided in Table 5.

Table 5. Full-text-verified species-specific ultrasound protocols and transfer limits

Commodity	Verified experimental protocol	Storage/context	Reported outcome	Transfer limitation
Strawberry (‘Benihoppe’)	28 kHz; 0.15 W cm^{-2}; 3 min; deionized water at 15 ± 1 °C.	5 ± 1 °C, 75–85% RH, up to 24 d.	Lower firmness loss and soluble pectin; lower PE/PG; altered hormone/ROS genes; later aroma study showed VOC effects.	Cultivar-, bath- and package-specific; decay and sensory replication required.
Blueberry	25 kHz; reported 400 W; 2 min.	Postharvest wash and storage assessed in the cited study.	Higher firmness and lignin; altered oxidative indicators, enzymes and transcriptome.	Delivered power density and cavitation field require clarification before transfer.
Sweet cherry	33 kHz; 60 W; 10, 20, 30, 40 or 60 min.	4 °C for 15 d.	20–40 min best overall; 40 min lowest microbial load; firmness injury emerged beyond 20 min.	Long treatment and apparatus dependence; must confirm cultivar and throughput feasibility.
Tomato (‘Zinac’)	45 kHz; 80% equipment power; 32–48 °C; 13–47 min; RSM optimisation.	10 °C for 1–15 d; selected 40 °C/30 min condition.	Delayed colour change, higher phenolics and acceptable overall quality.	Thermosonication; heat and ultrasound contributions are not interchangeable.
Pink guava	40 kHz; 150 W; 0.5% calcium gluconate; 2–8 min; 4 min selected.	27 ± 1 °C for 6 d.	Colour, firmness, membrane integrity and antioxidant attributes maintained.	Combined calcium–ultrasound process; component controls needed.
Winter jujube	28 kHz with or without 10 ppm free chlorine.	Whole ready-to-eat fruit; 4 °C for 7 d; inoculated pathogens.	Combination prevented detected cross-contamination; ultrasound alone did not.	Sanitizer residual, water organic load and validation organism determine performance.

Source: Zhang et al. (2022), Hou et al. (2024), Muzaffar et al. (2016), Pinheiro et al. (2019), Supapvanich et al. (2022), Wang et al. (2022), and Li et al. (2024).

5.7. Why the protocols remain candidates rather than prescriptions

The protocols in Table 5 were measured in specific cultivars, maturity states, baths, water volumes, equipment configurations and storage systems. Their quantitative parameters can be reproduced as reference conditions, but a species name alone is insufficient to justify direct commercial transfer (Jiang et al., 2020; Zhou et al., 2022).

The most defensible use of the table is to define starting points for replication and response-surface optimisation. A protocol becomes species-specific only after cultivar and maturity boundaries are known, and it becomes commercial only after realistic load, throughput, microbial, sensory, safety and economic criteria are met.

6. DESIGNING REPRODUCIBLE SPECIES-SPECIFIC PROTOCOLS

6.1. Commodity definition and biological unit

A protocol must state species, cultivar, production site or lot, maturity index, size distribution, defect status, pre-cooling history and time from harvest to treatment. Fruit within a tray or tank are subsamples, whereas the biological unit is usually an independently treated fruit, package, tank run or lot; confusing subsamples with independent replicates inflates apparent precision.

Randomization should occur before treatment, and blocking should be considered for harvest lot, treatment day, chamber or tank run. Repeated measurements on the same fruit require a longitudinal model or an explicitly non-destructive design rather than analysis as independent observations.

6.2. Acoustic metrology and sham controls

Airborne studies should report unweighted and, where relevant, frequency-weighted sound-pressure level at multiple product positions, background noise, speaker distance and orientation, waveform, harmonic distortion and vibration transmitted through shelves or trays. A sham chamber should reproduce temperature, airflow, handling and equipment heat without the target acoustic signal (Hassanien et al., 2014; Kim et al., 2018).

Ultrasound studies should report tank dimensions, water volume and depth, product load, transducer placement, nominal and delivered power, temperature trajectory, dissolved gas and water chemistry. Calorimetric power or another field-validation method is needed because electrical input and equipment percentage do not directly quantify acoustic energy reaching the commodity (Jiang et al., 2020; Zhou et al., 2022).

6.3. Factorial separation of co-treatments

When ultrasound is combined with heat, calcium, chlorine, peracetic acid, UV-C or coating, the minimum design is a factorial comparison of control, ultrasound alone, co-treatment alone and the combination. Without those groups, the combination can be evaluated as a process, but the contribution and interaction of its components cannot be estimated (Esua et al., 2020; Pinheiro et al., 2019; Supapvanich et al., 2022; Wang et al., 2022).

Bath temperature should be monitored continuously because ultrasound can heat the medium, and water controls should follow the same temperature–

time trajectory. A nominally non-thermal treatment becomes thermosonication when the temperature contributes materially to the response (Pinheiro et al., 2019).

6.4. Outcome hierarchy

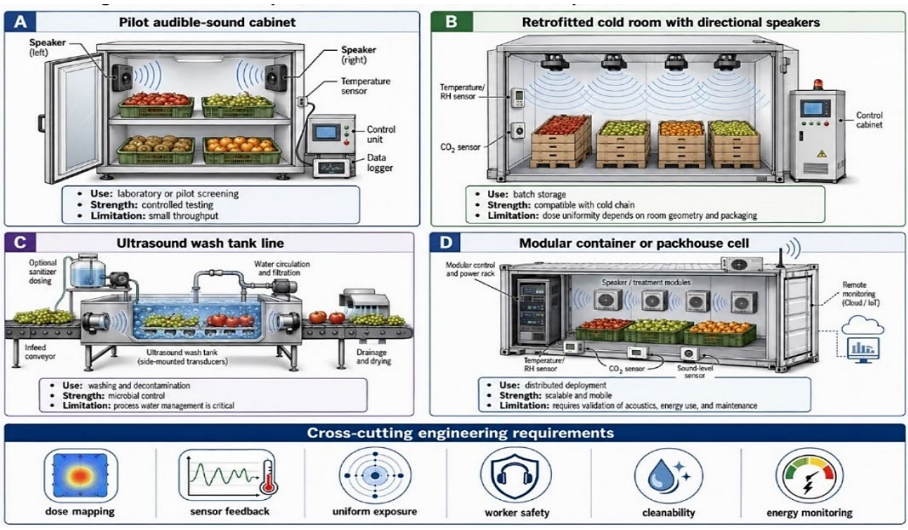
A primary outcome should be declared before analysis, such as ethylene production, firmness, decay incidence or a validated pathogen reduction. Secondary outcomes can include colour, respiration, mass loss, soluble solids, acidity, membrane leakage, antioxidant activity, aroma, sensory scores and molecular endpoints.

Molecular data should be interpreted in the context of phenotypic outcomes and corrected for multiple testing. Many differentially expressed genes does not by itself establish preservation efficacy, while a visible colour delay does not establish acceptable flavour, nutrition or retail performance (Kim et al., 2018; Zhang et al., 2022; Li et al., 2024).

6.5. Dose–response and adverse-effect boundary

Optimization should include a no-effect region, a beneficial region and an injury region. The cherry experiment provides direct evidence that prolonged exposure can reduce firmness and other quality attributes, and reviews of ultrasonic processing identify tissue damage, erosion and nutrient loss as potential adverse effects (Muzaffar et al., 2016; Jiang et al., 2020; Zhou et al., 2022).

An operating window should therefore be defined by both minimum effective dose and maximum tolerated dose. Reporting only the best treatment obscures the response surface and prevents risk-based scale-up.



Source: Author's original methodological framework informed by Kim et al. (2015, 2018), Jiang et al. (2020), Zhou et al. (2022), Zhang et al. (2022), and Hou et al. (2024).

Figure 4. Minimum experimental-design framework for reproducible postharvest-acoustics studies.

Minimum experimental-design framework for reproducible postharvest-acoustics studies is presented in Figure 4, and minimum reporting standard for postharvest sound and ultrasound experiments are provided in Table 6.

Table 6. Minimum reporting standard for postharvest sound and ultrasound experiments

Domain	Mandatory items	Reason
Commodity	Species, cultivar, maturity, lot, size, defects, harvest-to-treatment interval, pre-cooling and package.	Defines biological and supply-chain context.
Airborne acoustic dose	Frequency, waveform, SPL reference and weighting, speaker, distance, orientation, spatial map, background and structural vibration.	Allows reproduction of the pressure/vibration field.
Ultrasound dose	Frequency, mode, nominal and delivered power, tank geometry, water volume/depth, transducer position, load and field map.	Separates equipment settings from delivered exposure.
Medium and thermal history	Air temperature/RH/airflow or water temperature/pH/turbidity/dissolved gas/sanitizer; continuous temperature trace.	Controls propagation and thermal/chemical confounding.
Experimental design	Biological unit, sample size, randomization, blocking, sham and factorial co-treatment controls.	Prevents pseudoreplication and attribution errors.
Storage	Temperature, RH, atmosphere, package, duration, sampling times and retail simulation.	Defines the environment in which efficacy is claimed.
Outcomes	Predeclared primary endpoint; quality, physiology, microbiology, sensory and molecular methods with units.	Prevents selective reporting and supports practical interpretation.
Statistics	Model, repeated-measure handling, multiple-test correction, effect size, uncertainty and all adverse/null results.	Allows assessment of precision and robustness.
Data availability	Raw data, calibration files, acoustic maps, code and treatment log where possible.	Enables audit and independent replication.

Source: Author's synthesis from the reporting deficiencies and process variables documented in the cited primary studies and reviews.

7. TRANSLATION TO COMMERCIAL STORAGE AND PACKING SYSTEMS

7.1. Airborne cold-room architecture

A commercial airborne system would require a signal generator, calibrated amplifiers and speakers or shakers, distributed microphones or accelerometers, temperature and humidity sensors, and a logger that records actual exposure for each lot. Speaker counts and location must be based on a loaded-room map rather than an empty-room calculation because pallets, bins, liners and packages alter reflection and attenuation.

The key control problem is spatial uniformity. A room-average sound-pressure level can conceal local maxima and minima, and the biological response may depend on the tissue-level vibration rather than the nominal electrical setting; product positions must therefore be linked to measured dose and outcome.

The tomato reference protocol was performed in a soundproof chamber at room temperature, whereas commercial tomato handling may involve pre-ripening rooms, transport, packaging and different temperatures. The first pilot should reproduce the biological phenotype in a small, loaded chamber before testing large-room arrays or prolonged schedules (Kim et al., 2015, 2018).

7.2. Ultrasound wash-line architecture

A commercial ultrasound unit would normally be integrated with product feeding, immersion or spray, transducer arrays, recirculation, filtration, temperature control, sanitizer dosing where applicable, rinsing and dewatering. The system must maintain the validated acoustic and chemical field while product load and water quality change during production (Zhou et al., 2022; Wang et al., 2022).

Standing-wave patterns and shielding by baskets or dense product loads can create under-treated zones, while excessively intense zones can cause tissue injury or equipment erosion. Hydrophone or validated cavitation mapping, calorimetry and loaded-tank challenge tests are therefore process-development requirements rather than optional descriptive measurements (Jiang et al., 2020; Zhou et al., 2022).

7.3. Package, atmosphere and cold-chain interactions

Packaging can attenuate airborne pressure, change structural resonance and isolate fruit from the external field. In immersion systems, the need for direct liquid contact may conflict with packages that are applied before treatment or with commodities that cannot tolerate wet handling.

Controlled or modified atmospheres alter respiration and ethylene physiology and may interact with a sound-induced delay. A combined system should include treatment-only, atmosphere-only and combined groups because an additive or antagonistic interaction cannot be inferred from either technology separately.

Cold temperature changes ripening rate, membrane state, liquid properties and cavitation behavior. The room-temperature tomato phenotype and the cold-stored ultrasound protocols must therefore be validated within the exact temperature–atmosphere sequence intended for the supply chain (Kim et al., 2015; Jiang et al., 2020).

7.4. Microbial safety and wash-water management

A lower aerobic count is not equivalent to control of a defined pathogen, and pathogen removal from one fruit can contaminate another fruit or the wash water. Challenge studies should measure both produce reduction and cross-contamination under realistic organic load and water-reuse conditions (Wang et al., 2022).

When a sanitizer is used, free residual, contact time, pH, temperature and organic demand become critical process variables. The winter-jujube study shows that ultrasound can enhance a low-concentration sanitizer system, but it also shows that ultrasound alone may be inadequate for cross-contamination control (Wang et al., 2022).

7.5. Sensory quality and marketable life

Commercial success requires marketable-life extension rather than delay of a single laboratory indicator. Trials should include texture, colour, mass loss, soluble solids, acidity, aroma, flavour, visual defects, decay, consumer or trained-panel assessment, and a shelf phase after storage.

The strawberry aroma study demonstrates that ultrasound can alter volatile metabolism and that effects vary over storage time. A treatment that maintains firmness but suppresses desirable aroma, creates uneven ripening or

transfers losses to the retail phase should not be classified as successful (Li et al., 2024).

7.6. Energy, water, equipment and occupational safety

Scale-up should report electrical energy per tonne or tonne-day, throughput, cooling demand, water consumption, filtration and sanitizer demand, cleaning time, transducer or speaker maintenance and downtime. Biological efficacy without a process-cost denominator is insufficient for commercial comparison.

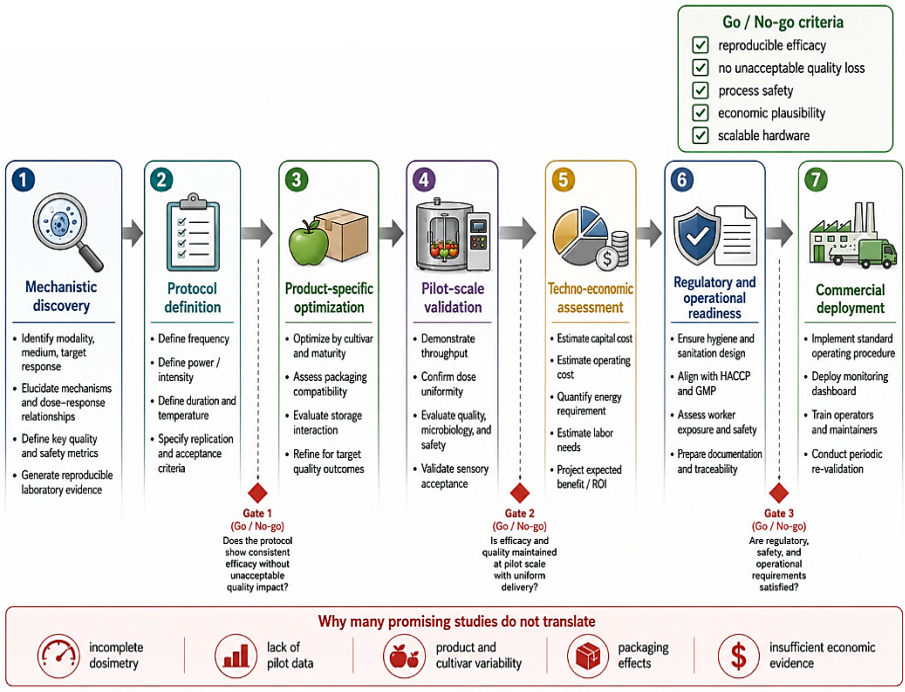
Airborne systems require assessment of worker exposure to audible and unintended subharmonic noise, and ultrasound systems require enclosure, maintenance and monitoring for audible components, cavitation noise and equipment erosion. Applicable occupational, electrical, hygiene and food-contact requirements depend on jurisdiction and must be assessed during plant design.

7.7. Economic comparison and decision criterion

A sound-based process should be compared with the relevant existing alternative for the same commodity, such as temperature management, 1-methylcyclopropene, controlled atmosphere, sanitizer washing, coating or another non-thermal treatment. The decision endpoint should be incremental marketable yield or quality value relative to total incremental cost, not acoustic energy consumption alone.

Economic analysis should include treatment capital, life, utilization, labour, maintenance, water, energy, cooling, quality losses, rejected lots and any premium or waste reduction. Because commercial efficacy has not been established for airborne tomato treatment, return-on-investment values should not be reported without actual pilot cost and loss data.

Commercial translation architectures and critical control points for airborne cold-room treatment and an ultrasound wash line are presented in Figure 5, and commercial readiness checklist and release criteria are provided in Table 7.



Source: Author's original engineering synthesis based on the experimental limitations in Kim et al. (2015, 2018), Jiang et al. (2020), Esua et al. (2020), Wang et al. (2022), and Zhou et al. (2022).

Figure 5. Commercial translation architectures and critical control points for airborne cold-room treatment and an ultrasound wash line.

Table 7. Commercial readiness checklist and release criteria

Readiness domain	Evidence required before progression	Example go/no-go criterion
Biological repeatability	Reference phenotype reproduced across independent lots and at least one independent laboratory.	Primary endpoint direction and minimum effect reproduced with prespecified analysis.
Dose uniformity	Loaded system mapped at all critical product positions.	Predetermined fraction of product positions within validated dose window.
Commodity boundary	Cultivar, maturity, size and defect limits tested.	SOP defines acceptable incoming product and rejects out-of-range lots.
Process interaction	Package, atmosphere, temperature and co-treatment controls completed.	No hidden dependence on an uncontrolled thermal, chemical or packaging factor.
Microbial safety	Relevant pathogen/surrogate and cross-contamination trial where a safety claim is intended.	Validated log reduction or cross-contamination limit under worst-case water/load conditions.
Sensory and retail performance	Storage plus retail simulation with sensory and marketability outcomes.	No unacceptable aroma, texture, colour or uneven-ripening penalty.
Equipment and worker safety	Noise, electrical, hygiene, erosion and maintenance assessment.	Compliance with applicable limits and documented preventive maintenance.
Economics	Capital and operating cost compared with the relevant alternative.	Positive value under conservative marketable-yield assumptions.
Routine verification	Calibration, treatment records, alarms, corrective action and traceability.	Every commercial lot has a verifiable exposure record and release decision.

Source: Author's commercial-validation framework; the entries are requirements for future validation rather than claims that the current literature has met them.

8. EVIDENCE GAPS AND PRIORITY EXPERIMENTS

8.1. Independent replication of airborne tomato treatment

The highest-priority experiment is a preregistered independent replication of the 1 kHz, 6 h mature-green tomato phenotype using the original cultivar and storage condition. The study should measure chamber SPL and tissue vibration, use an identical sham chamber, predefine ethylene or objective colour as the primary endpoint, and make the raw time-series data available (Kim et al., 2015, 2018).

A second phase should test cultivar and temperature interactions rather than immediately screening many unrelated species. Failure to replicate the reference phenotype would redirect research toward chamber-specific or lot-specific explanations, whereas successful replication would justify broader optimization.

8.2. Direct tests of the mechanotransduction hypothesis

Real-time calcium imaging, ROS reporters, electrophysiology or membrane-potential probes should be used in harvested pericarp or epidermal tissue during and after a calibrated acoustic exposure. Pharmacological controls and, where feasible, genetic lines affecting candidate mechanosensitive channels or calcium pathways should test necessity rather than association (Peyronnet et al., 2014; Mishra et al., 2016).

The experiment should distinguish airborne pressure from shelf vibration by using mechanically isolated fruit, structural shakers and matched pressure fields. This separation is necessary before a commercial designer can decide whether loudspeakers, contact actuators or another excitation system is most appropriate.

8.3. Ultrasound metrology and inter-laboratory transfer

The strawberry, blueberry, cherry, tomato, guava and jujube protocols should be repeated with delivered power measured by calorimetry or an equivalent method and with spatial cavitation maps under product load. Nominal watts, equipment percentage and frequency should be retained as equipment descriptors but not treated as complete dose measures (Jiang et al., 2020; Zhou et al., 2022).

Round-robin trials using the same commodity and treatment target in different baths would reveal whether the biological response transfers when

delivered energy is matched. Such trials are more informative for commercial standardization than accumulating unrelated single-bath commodity reports.

8.4. Dose-window models and multi-objective optimization

Future optimization should simultaneously model benefit and injury endpoints. For example, microbial reduction, firmness retention and antioxidant response can improve over part of the dose range while tissue leakage, softening or sensory defects worsen beyond a threshold, as illustrated by the cherry duration experiment (Muzaffar et al., 2016).

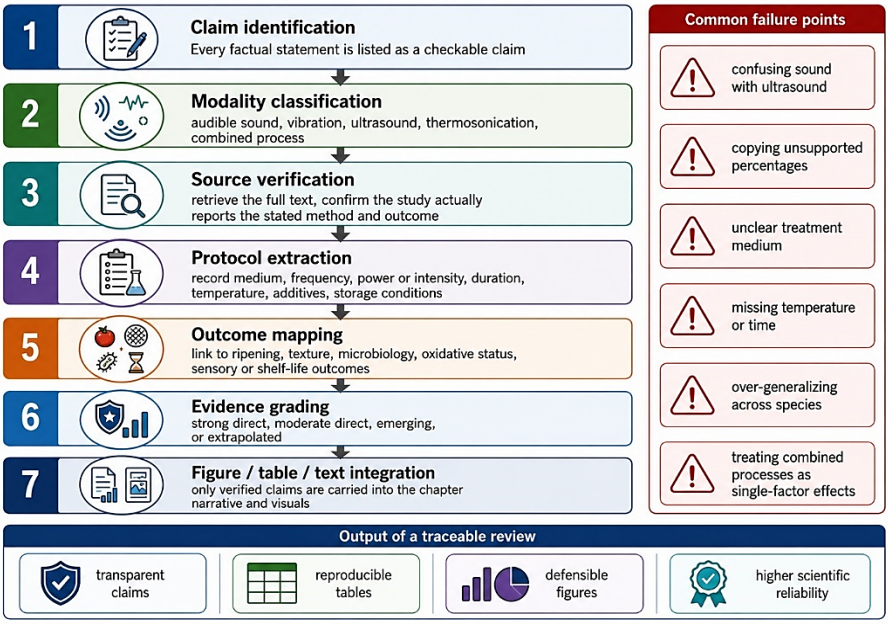
A multi-objective model should identify the Pareto region rather than a single optimum based on one outcome. The selected commercial set point should include a safety margin for load and equipment variability.

8.5. Pilot-scale economics and life-cycle variables

Pilot studies should record energy, water, sanitizer, cooling, labour, throughput, maintenance and product loss in parallel with biology. These data are required to compare a sound-based process with existing postharvest technologies and to avoid unsupported claims that acoustic treatment is intrinsically low-cost or low-energy.

Environmental assessment should consider water reuse, chemical reduction or increase, equipment manufacture, cooling load and avoided food loss. No single sustainability label is scientifically justified without a defined functional unit and comparator.

Stage-gated pathway from source verification to commercial qualification are presented in Figure 6.



Source: Author's original synthesis based on the evidence limitations and validation needs documented throughout this chapter.
Figure 6. Stage-gated pathway from source verification to commercial qualification.

9. INTEGRATED RECOMMENDATIONS BY MODALITY

9.1. Airborne audible sound

The only current candidate protocol that can be stated with substantial direct postharvest detail is 1 kHz for 6 h in mature-green 'Dotaerang' tomato under the reported chamber and room-temperature storage conditions. It should be reproduced exactly before the frequency, level, duration, cultivar or temperature is changed (Kim et al., 2015, 2018; Jeong et al., 2023).

The mechanistic working model should centre on ethylene and ripening regulation, while mechanosensitive channels, calcium and ROS remain explicitly labelled upstream hypotheses. Commercial-room design should not begin with a universal speaker layout; it should begin with an independent biological replication and a loaded-chamber dose map.

9.2. Power ultrasound

Power ultrasound has multiple commodity-specific starting conditions, but the selected condition must include the liquid and co-treatment context. The strawberry protocol is suitable for replication of texture and aroma outcomes,

the cherry protocol is suitable for studying duration–injury trade-offs, and the winter-jujube protocol is suitable for hurdle-based cross-contamination research (Muzaffar et al., 2016; Wang et al., 2022; Zhang et al., 2022; Li et al., 2024).

Thermosonication and calcium-assisted ultrasound should be retained as combined processes in terminology, experimental design and conclusions. Their outcomes cannot be used as evidence that an ultrasound-only treatment at the same frequency will perform similarly (Pinheiro et al., 2019; Supapvanich et al., 2022).

9.3. Acoustic sensing and adaptive control

Acoustic sensing can be developed independently as a non-destructive method for lot characterization and treatment verification. Its most valuable future role may be to stratify fruit by firmness or maturity and provide feedback to a treatment controller, but a closed-loop treatment–sensing system has not yet been validated in the studies reviewed here (Taniwaki & Sakurai, 2010).

CONCLUSIONS

The scientifically defensible evidence base is narrower than broad narrative summaries may suggest, but it provides a coherent basis for critical evaluation. Airborne audible sound has a coherent multi-omics proof of concept in harvested tomato, including physiological, transcriptional, non-coding RNA, metabolomic and epigenetic evidence, while independent cultivar, laboratory and commercial replication remain absent (Kim et al., 2015, 2018, 2023; Jeong et al., 2023).

Power ultrasound has broader commodity evidence and clearly defined candidate windows in strawberry, blueberry, sweet cherry, tomato, pink guava and winter jujube. Its effects depend on cavitation field, delivered power, time, temperature, water chemistry and co-treatments, and beneficial and damaging outcomes can occur within the same experimental range (Muzaffar et al., 2016; Pinheiro et al., 2019; Jiang et al., 2020; Wang et al., 2022; Zhang et al., 2022; Supapvanich et al., 2022; Hou et al., 2024; Li et al., 2024).

Species-specific protocols should therefore be treated as fully specified and locally validated operating windows rather than frequency labels. Every future protocol should identify the commodity and maturity, measure the acoustic field, separate co-treatments, define a primary biological endpoint,

report injury and null effects, and test packaging, atmosphere, sensory quality, microbial safety and economics under realistic load.

The route to commercial storage is feasible as a research programme but not yet established as a universal technology. Progress will depend less on discovering additional isolated frequencies and more on independent replication, acoustic metrology, causal physiology, process validation and transparent claim-to-source traceability.

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CHAPTER 16

AI-BASED GRAPEVINE IDENTIFICATION AND EARLY DISEASE DETECTION: A DIGITAL AGRICULTURE FRAMEWORK USING DEEP LEARNING AND REMOTE SENSING

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1. INTRODUCTION

Grapevine (Vitis vinifera L. and related Vitis spp.) is a high-value perennial crop in which biological identity and plant health are tightly linked to management. Correct cultivar identification is important for germplasm conservation, nursery certification, vineyard establishment and traceability, whereas disease surveillance is important for protecting yield and quality. Conventional ampelography depends on expert interpretation of morphological descriptors, while laboratory diagnosis can be difficult to scale across large vineyard areas. Digital agriculture provides a complementary route by converting images, sensor data and spatial observations into georeferenced, reproducible and increasingly explainable information. (OIV, 2023; Güzel, 2026)

Current vineyard-health research increasingly draws on several observation scales rather than relying on a single camera or sensor. Field studies now combine colour imagery with multispectral or hyperspectral measurements, spectroscopy and aerial platforms, allowing plant-level observations to be interpreted alongside spatial patterns across the vineyard. A systematic review of 104 field studies published from 2008 to October 2024 identified RGB, multispectral, hyperspectral and field-spectroscopy methods as major sensing families and highlighted multi-sensor integration, methodological standardisation and validation across diverse vineyards as priorities. (Portela et al., 2024)

This chapter treats cultivar identification and disease surveillance as connected rather than isolated digital tasks. Cultivar identity can provide biological context for health monitoring, while disease and abiotic stress can alter the leaf characteristics used in cultivar recognition. The proposed framework therefore links digital ampelography, field imaging, remote sensing, environmental observations and GIS/GeoAI within a human-in-the-loop decision-support architecture. (Güzel et al., 2024; Portela et al., 2024)

2. DIGITAL AMPELOGRAPHY AS THE BIOLOGICAL IDENTITY LAYER

2.1 From classical descriptors to image-based recognition

Ampelography describes grapevine varieties using morphological characteristics of shoots, leaves, clusters and berries. For mature leaves, shape, lobing, sinus characteristics, teeth and other descriptors provide structured biological information. Digital image analysis can quantify these features or learn discriminative representations directly from pixels, while explainability tools can be used to inspect whether model attention corresponds to plausible ampelographic regions. (OIV, 2023; Selvaraju et al., 2017)

2.2 Author case study: hybrid DenseNet-machine-learning pipeline

The technical case study used in this chapter comprises 4,186 high-resolution leaf images from 20 economically important cultivars, divided into 10 table and 10 wine cultivars and including both adaxial and abaxial leaf surfaces. The case study was developed as part of the author's doctoral research at Van Yüzüncü Yıl University. DenseNet121, DenseNet169 and DenseNet201 were evaluated as deep feature extractors (Huang et al., 2017). CBAM and Squeeze-and-Excitation attention modules were examined (Woo et al., 2018; Hu et al., 2018), and feature-selection strategies included SelectKBest/ANOVA-F, principal component analysis (PCA), recursive feature elimination (RFE) and SHAP-based selection. The downstream classifiers evaluated in the current study include Logistic Regression, support vector machines, Random Forest, XGBoost and Naive Bayes, with performance assessed using 5-fold stratified cross-validation.

2.3 Performance and explainability

In the current author dataset, the best wine-grape configuration (DenseNet169 + RFE + Random Forest) achieved 99.62% accuracy. The best table-grape configuration (DenseNet201 + Logistic Regression) reached 98.21%, and the combined 20-class task also yielded 98.21% accuracy. These values match the latest version of the author manuscript used for this chapter. They are reported here as results from the author case study and should not be interpreted as external benchmark values.

Grad-CAM can provide spatial heat maps indicating image regions that influence convolutional-network predictions, while SHAP provides feature-level attribution for model outputs. In the author case study, the Grad-CAM visualisations indicate attention in leaf regions that are biologically plausible for ampelographic discrimination; these visualisations should be interpreted as model-explanation evidence rather than as proof of causal morphological features. (Selvaraju et al., 2017; Lundberg & Lee, 2017)

3. AI FOR GRAPEVINE DISEASE DETECTION

3.1 Disease targets and imaging signals

Grapevine diseases differ in symptom expression and sensing scale. Downy mildew and powdery mildew may produce visible foliar symptoms; gray mold can affect berries and other tissues; trunk-disease complexes and viral or yellowing disorders can alter canopy appearance and reflectance. RGB imaging is inexpensive and useful when symptoms are optically visible, whereas multispectral, hyperspectral, thermal and spectroscopy-based methods can provide additional physiological or spectral evidence. (Portela et al., 2024; Güzel, 2026)

3.2 Deep learning for image-based diagnosis

Deep learning has been used for grapevine health recognition through transfer learning, hybrid neural architectures and dedicated leaf-disease classifiers. These studies demonstrate strong image-classification performance, but results obtained from curated or controlled image datasets should not be interpreted as direct evidence of equivalent performance under commercial field conditions. (Elsherbiny et al., 2024; Talaat et al., 2025)

3.3 Controlled early detection versus field detection

A controlled experiment by Hernández, Gutiérrez, and Tardaguila (2024) examined Tempranillo leaf discs photographed in RGB after inoculation with downy mildew. Their results indicate that the infected and healthy samples could already be separated at the third day after inoculation, when the reported classification performance was about 99% accuracy with an F1-score of 0.99. The same study reported 81% accuracy and an F1-score of 0.77 for infection-stage identification. Because the images were acquired from leaf discs in Petri dishes under controlled conditions, these results should not be described as

commercial-vineyard pre-symptomatic validation. (Hernández, Gutiérrez, & Tardaguila, 2024)

A separate study addressed a much more demanding field setting. Hernández, Gutiérrez, Barrio, et al. (2024) analysed canopy imagery obtained from 14 commercial vineyard plots to determine whether downy-mildew symptoms could be both recognised and spatially localised. Their workflow achieved 91% classification accuracy, an F1-score of 0.92 and a localisation IoU of 0.83. A subsequent semantic-segmentation study using high-resolution canopy images from 14 vineyard plots reported 86% accuracy and an F1-score of 82% for classifying areas with and without symptoms, with an NRMSE of 12% for symptom counts. (Hernández, Gutiérrez, Barrio, et al., 2024; Hernández et al., 2025)

3.4 Multispectral and multimodal disease intelligence

For multispectral disease mapping, Kapetas et al. (2026) assembled 451 observations under open-field conditions. Each capture included conventional RGB information together with five spectral bands centred at 460, 540, 640, 780 and 880 nm. Their YOLOv11 and dual-head SegFormer workflow used multispectral channels, vegetation indices, segmentation masks and depth information. The SegFormer model achieved a mean IoU of 75.22% and an overall F1-score of 83.53%; disease-specific IoU reached 94.32% for gray mold and 75.5% for downy mildew. The authors positioned the approach for UAV and ground-robotics applications. (Kapetas et al., 2026)

Kotaidou et al. (2026) explored a different form of data fusion by analysing meteorological variables together with visual information from grapes and leaves. The model was designed to estimate expert-defined risk categories for gray mold, powdery mildew and downy mildew. The study supports multimodal risk estimation, but its validation is described by the authors as in vitro; it should therefore be cited as evidence for multimodal modelling rather than as proof of fully operational vineyard forecasting. (Kotaidou et al., 2026)

Field spectroscopy offers an additional route for detecting disease-related changes without relying on colour imagery alone. Portela et al. (2026) studied Vitis vinifera cv. Loureiro using spectra measured on 600 leaves sampled over the 2023 and 2024 seasons, and evaluated both PLS-LDA and

Random Forest classifiers. Both models achieved 95.1% overall accuracy when all spectral features were used, and the visible and red-edge regions contributed strongly to discrimination. (Portela et al., 2026)

Table 1. Verified recent evidence for AI-assisted grapevine disease monitoring

Study	Target	Setting/data	Approach	Verified result	Interpretation
Hernández, Gutiérrez, & Tardaguila (2024)	Downy mildew	Controlled leaf discs; RGB	CNN + Grad-CAM	~99% accuracy; F1 0.99 from day 3 post-inoculation	Controlled early-detection evidence; not field validation
Hernández, Gutiérrez, Barrio, et al. (2024)	Downy mildew	14 commercial vineyard plots; canopy RGB	CNN/ViT + XAI/localisation	91% accuracy; F1 0.92; IoU 0.83	Field detection and localisation
Hernández et al. (2025)	Downy mildew	14 vineyard plots; high-resolution canopy images	Semantic segmentation	86% accuracy; F1 82%; symptom-count NRMSE 12%	Field segmentation; real-time potential
Kapetas et al. (2026)	Downy mildew; gray mold	451 open-field multispectral captures	YOLOv11 + dual-head SegFormer	mIoU 75.22%; gray mold IoU 94.32%; downy mildew IoU 75.5%	Multi-sensor segmentation for robotic/UAV use
Portela et al. (2026)	Downy mildew	600 field leaves; spectroscopy	PLS-LDA + RF	95.1% overall accuracy	Field spectroscopy across two seasons
Kotaidou et al. (2026)	Gray mold; powdery/downy mildew	Weather + grape/leaf images	Multimodal deep learning	Promising expert-defined risk estimation	Multimodal concept; validation described as in vitro

Source: Hernández, Gutiérrez, & Tardaguila (2024); Hernández et al. (2024, 2025); Kapetas et al. (2026); Portela et al. (2026); Kotaidou et al. (2026).

Note. Results are reported only at the level supported by the cited publications; controlled laboratory, field and in vitro evidence are deliberately distinguished. Complete bibliographic records and DOI links are provided in the References section.

4. REMOTE SENSING, UAVS AND GEOAI FOR VINEYARD SURVEILLANCE

Leaf-level observations are valuable but cannot by themselves describe spatial variability across a commercial vineyard. UAV and satellite observations can provide a spatial screening layer for canopy condition and anomalous zones. Vegetation indices, canopy temperature, high-resolution RGB imagery and other spatial features can be integrated with parcel

boundaries, topography, weather and field observations in a GIS. Previous grape-growing spatial decision-support work has demonstrated the value of combining climate, soil and topographic variables within GIS for viticultural decisions. (Güzel et al., 2024)

Across the field literature reviewed by Portela et al. (2024), UAV-based acquisition appears frequently among remote-sensing approaches, while machine-learning and deep-learning methods are increasingly used to interpret the resulting observations. However, spectral anomalies are not necessarily pathogen-specific: nutrient limitation, water stress, canopy structure, illumination and other factors can produce similar responses. Remote sensing is therefore most defensible as a screening and prioritisation layer connected to proximal observations and expert ground truth. (Portela et al., 2024)

Hyperspectral imaging from UAVs has also been evaluated for viral disease surveillance. In the Australian vineyard study of Wang et al. (2024), the best reported prediction accuracy reached 98%, and the authors also tested the approach across different locations and seasons. The 'up to 98%' value should be interpreted as the best reported performance in that study rather than a universal accuracy for all cultivars or conditions. (Wang et al., 2024)

5. INTEGRATED CULTIVAR-AWARE SMART-VITICULTURE FRAMEWORK

The proposed architecture connects biological identity with plant-health and spatial context. Cultivar identity is not treated as a deterministic disease-susceptibility label; instead, it is used as one contextual variable alongside symptom probabilities, environmental conditions and spatial observations. This avoids the unsupported assumption that cultivar identity alone can diagnose disease while still recognising that cultivar morphology, phenology and canopy structure can influence image appearance and risk interpretation.

Table 2. Layers of the proposed cultivar-aware vineyard intelligence framework

Layer	Primary data	Analytics	Decision output
Biological identity	Leaf RGB images	DenseNet + attention + ML + XAI	Cultivar identity and confidence
Proximal health	RGB/multispectral/thermal leaf or canopy data	Classification, detection or segmentation	Disease/stress evidence
Aerial/spatial	UAV/satellite imagery	Indices, anomaly detection, GeoAI	Hotspot/risk maps
Environmental context	Weather, leaf wetness, soil, phenology	Time-series/risk modelling	Infection-favourability context
Decision support	Fused geospatial database	Multimodal fusion + rules/XAI	Scouting and expert-confirmation priorities

Source: Author synthesis prepared for this chapter from the cited digital-ampelography, remote-sensing and GeoAI literature.

5.1 End-to-end operational workflow

1. **Field acquisition:** Smartphone, fixed camera or UAV platforms capture geotagged leaf/canopy imagery.
2. **Quality control:** Automated checks flag blur, extreme illumination, occlusion and insufficient leaf/canopy coverage.
3. **Cultivar inference:** The digital-ampelography component estimates cultivar identity and confidence.
4. **Disease screening:** Detection or segmentation models estimate symptom class, location and severity.
5. **Context retrieval:** GIS retrieves parcel, cultivar map, topography, recent weather and available remote-sensing layers.
6. **Risk fusion:** A multimodal model combines image evidence, biological context, weather and spatial indicators.
7. **Explainability:** Grad-CAM, SHAP or segmentation overlays communicate evidence underlying predictions.
8. **Decision support:** The system returns scouting priority and risk category rather than automatically prescribing pesticide use.
9. Expert feedback: Confirmed observations are returned to the database for local calibration and future model improvement. The complete operational sequence is summarised in Figure 1.

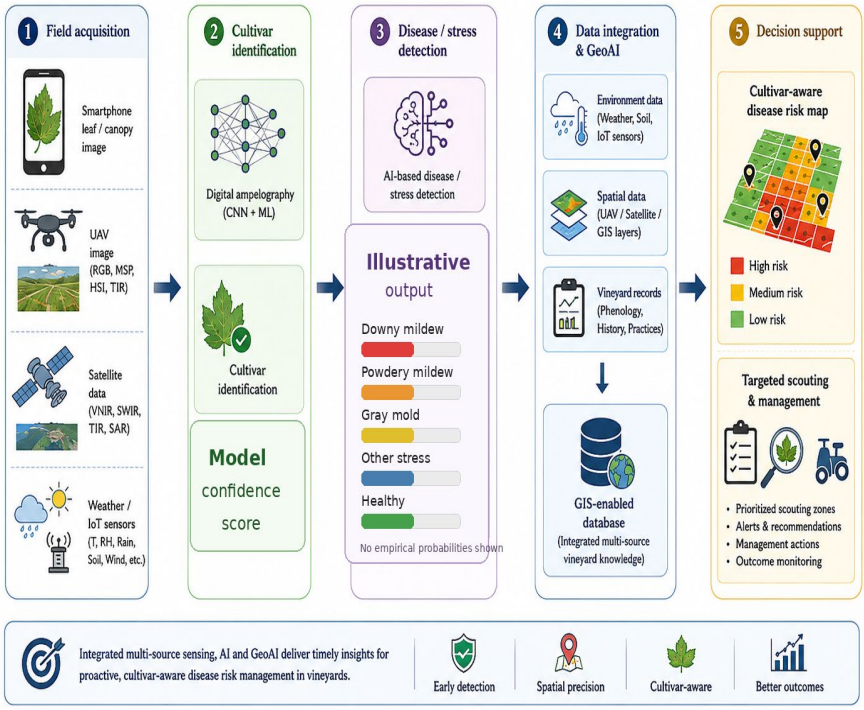

Figure 1. Operational workflow of the proposed cultivar-aware digital vineyard intelligence system.
Source: Conceptual synthesis prepared for this chapter; the diagram contains no empirical disease-probability values.

6. FROM RGB IMAGES TO MULTISENSOR VINEYARD INTELLIGENCE

6.1 RGB imaging

RGB cameras are the most accessible sensing technology in digital viticulture. Smartphones, conventional cameras, mobile platforms and UAVs can acquire colour imagery at relatively low cost. RGB images can support cultivar recognition and detection of visible symptoms, but they mainly capture changes once those changes become optically distinguishable in the visible spectrum. (Elsherbiny et al., 2024; Hernández et al., 2025)

6.2 Multispectral imaging

Multispectral imaging records reflectance in selected wavelength bands and can support vegetation indices and other descriptors related to canopy

vigour and physiological disturbance. Open-field multispectral work on downy mildew and gray mold demonstrates that combining spectral bands with derived indices and depth information can improve disease-specific segmentation. (Kapetas et al., 2026)

6.3 Hyperspectral imaging and field spectroscopy

By sampling reflectance in many adjacent narrow bands, hyperspectral systems preserve far more spectral detail than conventional RGB or typical multispectral cameras. This richer signal can reflect changes related to pigment composition, tissue structure and plant water relations. Their richer information can support subtle stress discrimination, although cost, calibration and high dimensionality remain practical constraints. UAV hyperspectral imagery has achieved strong viral-disease discrimination in vineyards, and field spectroscopy has achieved 95.1% overall accuracy for downy-mildew classification in a two-season Loureiro dataset. (Wang et al., 2024; Portela et al., 2026)

6.4 Thermal imaging

Thermal infrared imaging measures leaf or canopy temperature and can reflect changes in transpiration and plant water relations. Thermal responses are not disease-specific, because water deficit, solar exposure and soil-moisture variation can produce similar temperature patterns. Thermal observations are therefore best interpreted together with visible/spectral imagery, weather data and field inspection. (Portela et al., 2024)

6.5 UAV and satellite observations

UAVs provide a high-resolution observational scale between individual plants and satellites and can carry RGB, multispectral, hyperspectral or thermal sensors. Satellite observations provide wider repeated coverage but generally at lower spatial resolution. A practical multiscale strategy can therefore use satellite data for broad screening, UAVs for vineyard-scale mapping and proximal imaging for vine- or leaf-level confirmation. (Portela et al., 2024) A comparative synthesis is provided in Table 3 and the multiscale architecture is illustrated in Figure 2.

Table 3. Comparison of sensing technologies for vineyard monitoring

Technology	Information domain	Main advantage	Main limitation	Typical role
RGB imaging	Visible colour and morphology	Low cost; fine visual detail	Primarily captures visible symptoms	Cultivar and symptom imaging
Multispectral imaging	Selected visible/NIR bands and indices	Compact spectral contrast; UAV compatible	Fewer bands; stress signals may lack disease specificity	UAV screening and disease/stress mapping
Hyperspectral / field spectroscopy	Many narrow spectral bands	Detailed spectral signatures	Cost, calibration and data complexity	Advanced disease/stress discrimination
Thermal imaging	Leaf/canopy temperature	Physiological stress evidence	Low etiological specificity	Complementary stress assessment
UAV platform	Carrier for RGB, multispectral, hyperspectral or thermal sensors	Flexible vineyard-scale mapping	Weather, flight logistics and regulation	Precision scouting and monitoring
Satellite observations	Repeated wide-area remote sensing	Regional and temporal coverage	Often coarser than proximal/UAV observations	Regional screening and change monitoring
Weather / IoT sensors	Temperature, RH, rainfall, soil moisture, leaf wetness	Environmental disease-risk context	No direct symptom observation	Risk modelling and validation
GIS / GeoAI	Spatiotemporal integration of multiple layers	Data fusion and risk mapping	Depends on data quality and interoperability	Decision support and prioritisation

Source: Author synthesis based on Portela et al. (2024), Wang et al. (2024), Kapetas et al. (2026), Portela et al. (2026), and the sensing literature cited in the text.

Note. Technology characteristics are presented as a qualitative synthesis rather than fixed sensor specifications; performance and spatial scale depend on platform, sensor and acquisition design.

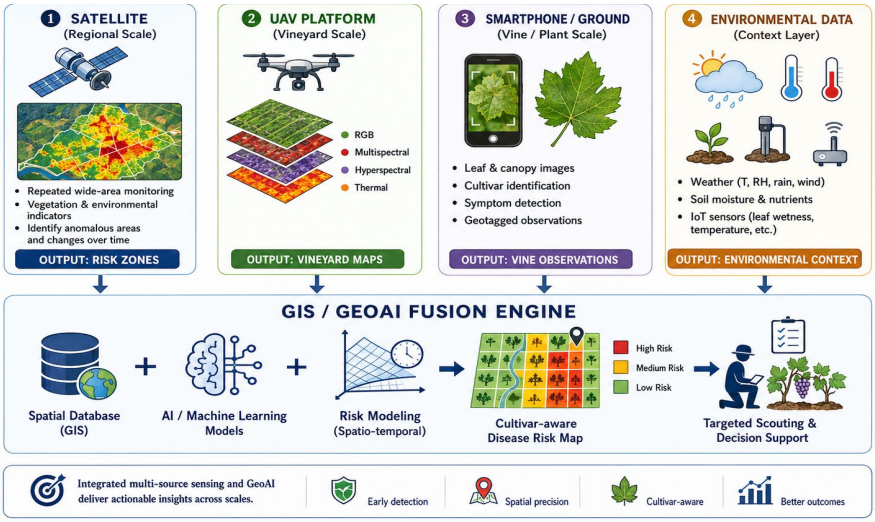

Figure 2. Multiscale and multisensor vineyard intelligence architecture integrating satellite, UAV, ground observations and environmental context within a GIS/GeoAI workflow.

Source: Conceptual synthesis prepared for this chapter based on Portela et al. (2024) and the recent multisensor studies cited in the text.

7. MULTIMODAL DATA FUSION AND GEOAI

No single sensor is likely to provide every piece of information needed for vineyard health assessment. A more useful representation is to describe each vine or block through several complementary variables, such as cultivar identity, image-based symptom evidence, spectral indicators, canopy temperature, recent weather, soil and terrain attributes, location and previous field observations. Multimodal modelling can fuse complementary evidence, while GIS/GeoAI can preserve the spatial and temporal relationships among observations. (Güzel et al., 2024; Kotaidou et al., 2026)

Multimodal interpretation is especially important because a visually or spectrally abnormal region is not necessarily caused by a pathogen. Similar signals can arise from water stress, nutrient deficiency, physical injury or canopy effects. Accordingly, risk maps should be used to prioritise field inspection and expert confirmation rather than as stand-alone etiological diagnoses. (Portela et al., 2024)

8. CULTIVAR-AWARE EARLY DISEASE INTELLIGENCE

Most disease-detection pipelines classify plant health without explicitly incorporating cultivar identity. The framework proposed here treats cultivar identity as contextual information rather than as a disease label. This may help distinguish natural morphological variability from abnormality and may support longitudinal traceability when cultivar identity, location and health observations are stored together.

8.1 Two-stage architecture

Stage 1 estimates cultivar identity and confidence from leaf imagery. Stage 2 evaluates the same or an additional image for disease/stress evidence. The resulting probabilities are passed to a contextual risk layer that can include phenology, temperature, humidity, rainfall, leaf wetness, prior disease observations and geospatial indicators. A suspicious image supported by favourable infection conditions and nearby anomalies can be prioritised for inspection, whereas unsupported or low-confidence observations can be flagged as uncertain.

8.2 Biological caution

The proposed cultivar-aware link is a research hypothesis for decision support, not evidence that the current cultivar-recognition model improves disease diagnosis. That added value should be tested prospectively by comparing cultivar-aware and cultivar-agnostic models on multi-cultivar, multi-location disease datasets. The conceptual relationships among identity, health, environmental, historical and spatial data are shown in Figure 3.

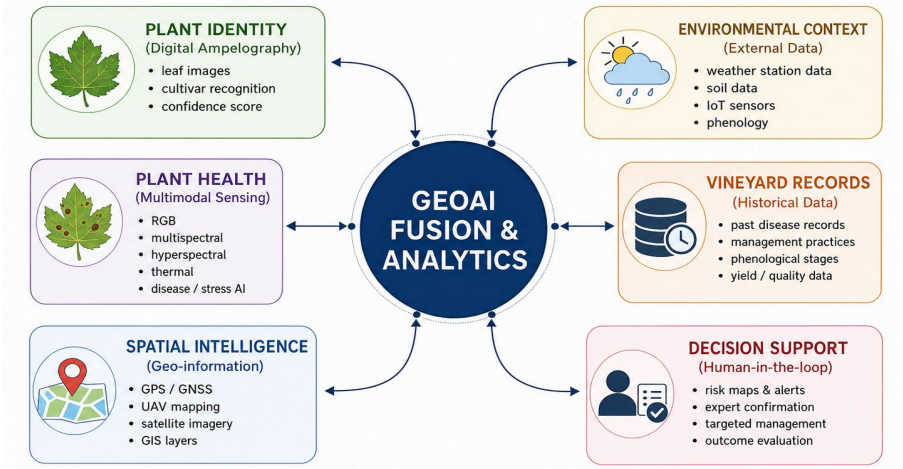

Figure 3. Cultivar-aware digital vineyard intelligence platform centred on GeoAI fusion and human-in-the-loop decision support.

Source: Conceptual synthesis prepared for this chapter; it represents a proposed architecture, not a validated deployed system.

9. EXPLAINABLE AI AND BIOLOGICAL INTERPRETATION

High predictive accuracy is insufficient if a model relies on background artefacts or non-biological correlations. Grad-CAM uses gradients to produce class-discriminative localisation maps for CNN predictions, while SHAP assigns feature-level contribution values within an additive explanation framework. These methods provide different but complementary views of model behaviour. (Selvaraju et al., 2017; Lundberg & Lee, 2017)

In digital ampelography, plausible spatial attention would be expected near leaf margins, teeth, sinuses, lobes, petiolar regions and venation-related structures described in ampelographic systems. For disease models, explanation maps should correspond to symptomatic tissue rather than to shadows, soil, trellis structures or unrelated foliage. Such plausibility checks do not prove causality, but they can reveal obvious model shortcuts and support expert review. Representative Grad-CAM outputs from the author's cultivar-identification study are shown in Figure 4. (OIV, 2023; Selvaraju et al., 2017)

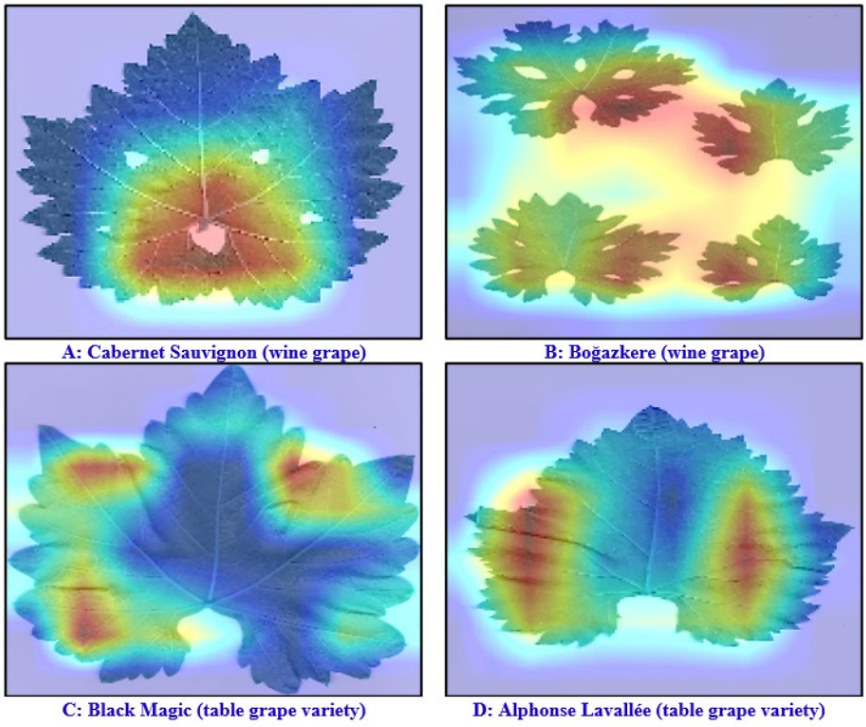

Figure 4. Representative Grad-CAM heatmaps for Cabernet Sauvignon, Boğazkere, Black Magic and Alphonse Lavallée in the author cultivar-identification study.
Source: Author dataset / current cultivar-identification manuscript; these are empirical model visualisations from the author study.

10. VALIDATION STRATEGY AND FIELD DEPLOYMENT

A credible field system should be evaluated at algorithmic, agronomic and operational levels. Algorithmic validation should report class-wise precision, recall and F1-score together with calibration and localisation/segmentation metrics where relevant. Agronomic validation should compare predictions with expert assessment and laboratory confirmation when required. Operational validation should test whether the system improves scouting efficiency, detection timing or spatial targeting under realistic vineyard workflows.

Data splitting must minimise information leakage. Images from the same vine, plot or acquisition session should not be distributed across training and test sets in ways that allow near-duplicate conditions to inflate performance.

Stronger designs include geographically independent test vineyards, leave-one-vineyard-out evaluation and multi-season validation. These requirements are consistent with the field-validation gaps identified in the systematic review literature. (Portela et al., 2024)

Performance obtained from controlled experiments, curated image collections and commercial-vineyard observations should be reported as distinct evidence categories. The day-3 early-detection result of Hernández, Gutiérrez, and Tardaguila (2024), for example, is controlled laboratory evidence, whereas the 2024 and 2025 canopy studies provide field-oriented evidence. This distinction prevents an accuracy value obtained in one acquisition domain from being misrepresented as an operational field estimate. (Hernández, Gutiérrez, & Tardaguila, 2024; Hernández, Gutiérrez, Barrio, et al., 2024; Hernández et al., 2025)

11. HUMAN-IN-THE-LOOP DECISION SUPPORT AND IMPLEMENTATION ROADMAP

Agricultural AI is best positioned here as a screening and prioritisation system. Instead of automatically prescribing treatment, the system should answer practical questions such as where the expert should inspect first, which observations require confirmation and where repeated monitoring is warranted. This is particularly important when visual or spectral symptoms have multiple possible biological causes. (Portela et al., 2024)

Table 4. Practical implementation roadmap

Stage	Main task	Minimum output	Readiness criterion
1	Adapt cultivar model to field imagery	Geotagged field leaf dataset + baseline model	Stable external-site cultivar performance
2	Add visible disease screening	Expert-annotated field dataset + detector/segmenter	Reliable class-wise field performance
3	Integrate UAV/satellite and weather	GIS database + spatial risk layers	Repeatable plot-level hotspot mapping
4	Fuse modalities	Cultivar + image + weather + geospatial model	Demonstrated gain over single-modality baselines
5	Deploy prototype	Mobile/web interface with XAI and maps	Successful expert/grower field trial

Source: Author-prepared implementation roadmap for the proposed cultivar-aware vineyard intelligence framework.

12. SUSTAINABILITY AND FARM-LEVEL VALUE

Integrated AI and sensing can contribute to sustainable management when information changes field actions rather than simply generating

additional maps. Potential applications include prioritised scouting, targeted sampling, identification of anomalous vines or blocks and improved spatial traceability. The author's previous work has also shown how GIS-based decision support can integrate multiple environmental criteria for grape cultivation, illustrating the broader value of spatially explicit decision frameworks in viticulture. (Güzel et al., 2024)

Claims about reduced pesticide use or economic benefit should nevertheless be treated as expected decision-support benefits until they are demonstrated through operational trials. Future validation should therefore measure management outcomes such as scouting time, intervention targeting, detection lead time, costs and environmental indicators rather than reporting classification accuracy alone. (Portela et al., 2024; Portela et al., 2026)

13. CHALLENGES AND LIMITATIONS

Domain shift and field variability. Models trained under controlled conditions may lose performance when transferred to new vineyards, seasons, cameras, illumination or canopy structures.

Limited and imbalanced labelled data. Reliable disease labels require expert assessment and, for some disorders, laboratory confirmation; rare or early stages can be underrepresented.

Symptom similarity and mixed stress. Chlorosis, necrosis, discoloration and canopy decline can arise from pathogens or abiotic constraints, limiting etiological certainty from imagery alone.

Sensor interoperability and calibration. RGB, multispectral, hyperspectral and thermal sensors differ in spatial resolution, spectral response, calibration and acquisition geometry.

Ground-truth quality. Disease confirmation, cultivar identity, phenology and management history should be recorded consistently to reduce label noise.

Explainability and trust. Explanation tools can reveal model attention but should be interpreted with expert biological review rather than treated as proof of causality.

Operational and economic constraints. UAV operations, specialist sensors, storage and analysis can increase complexity; adoption depends on measurable decision value.

Data governance and standardisation. Scalable systems require consistent metadata, interoperable formats and clear rules for georeferenced agricultural data.

These limitations closely match the priorities identified by the 2024 systematic review, particularly the need for methodological standardisation, multi-sensor integration and validation across diverse vineyard contexts. (Portela et al., 2024)

14. FUTURE RESEARCH DIRECTIONS

Multi-location and multi-season validation. Test cultivar and disease models in geographically independent vineyards and across multiple seasons.

Cultivar-aware disease modelling. Directly compare cultivar-aware and cultivar-agnostic models to determine whether cultivar information improves detection, severity estimation or risk forecasting.

Early and pre-symptomatic sensing. Evaluate whether spectral, thermal or physiological observations provide robust lead time before visible symptoms under field conditions.

Multimodal fusion. Develop models that combine image, spectral, weather and spatial observations while preserving uncertainty and temporal context.

Edge and mobile deployment. Evaluate efficient cultivar-recognition and symptom-screening models on field-capable devices and connect them to server-side GIS/GeoAI when appropriate.

Active learning and continuous improvement. Prioritise uncertain cases for expert annotation and use confirmed observations for controlled model updates.

Spatiotemporal vineyard records. Link identity, phenology, disease history, sensor observations and management actions over time.

Decision-support validation. Measure operational outcomes including scouting efficiency, detection lead time, intervention targeting, costs and environmental impact.

Recent field spectroscopy, hyperspectral UAV and multimodal studies provide a technically credible basis for these directions, but none removes the need for independent external validation in the intended vineyard population. (Wang et al., 2024; Kotaidou et al., 2026; Portela et al., 2026)

15. CONCLUSIONS

The direction of digital viticulture is shifting from stand-alone recognition models toward decision systems that combine plant identity, health observations, environmental information and geospatial context. The author case study shows that a hybrid DenseNet-machine-learning pipeline can achieve high cultivar-identification performance on a 20-cultivar leaf-image dataset, while published field studies demonstrate that AI can detect and localise grapevine disease symptoms under increasingly realistic conditions.

The proposed cultivar-aware framework links RGB, multispectral, hyperspectral and thermal observations with UAV/satellite remote sensing, weather and GIS/GeoAI. Its central contribution is conceptual: disease surveillance should be treated as a context-dependent decision-support problem rather than as an isolated image-classification task. Cultivar identity can provide biological context, but its added predictive value for disease intelligence remains a hypothesis that requires direct validation.

Operational deployment will require independent multi-vineyard and multi-season validation, reliable ground truth, uncertainty-aware outputs, explainability, interoperable sensing workflows and economic assessment. In the near term, the most defensible systems are human-in-the-loop tools that prioritise scouting and expert confirmation rather than autonomous treatment decisions. (Portela et al., 2024; Portela et al., 2026)

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CHAPTER 17

SELENIUM AND NANO-SELENIUM APPLICATIONS IN VEGETABLE CROPS UNDER DROUGHT AND SALINITY STRESS: FROM MECHANISMS TO PRACTICE

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1. INTRODUCTION

1.1. Global Climate Change and Stress Factors in Vegetable Production

Global climate change is among the most important environmental challenges of the twenty-first century and is profoundly affecting agricultural production systems (IPCC, 2021). Rising temperatures, altered precipitation patterns, and the increasing frequency of extreme weather events are causing serious yield losses, particularly in vegetable production (Dias, 2019). Although vegetables are indispensable components of the human diet, they are generally highly sensitive to abiotic stresses (El-Ramady et al., 2023). Among these constraints, drought and salinity are two of the most important factors limiting agricultural productivity (Munns and Tester, 2008; Farooq et al., 2009).

One billion hectares of land worldwide is affected by several kinds of salinity and associated problems (Basak et al., 2022). Drought is likewise one of the most destructive natural hazards threatening vegetable production, particularly in arid and semi-arid regions (Farooq et al., 2009). Although drought and salinity induce several similar physiological and biochemical disturbances, their primary modes of action differ.

1.2. Physiological and Biochemical Effects of Drought Stress on Vegetable Crops

Drought stress is a complex process initiated by a decline in soil water potential and followed by a cascade of physiological responses (Farooq et al., 2009). Its earliest and most evident effect is a reduction in plant tissue water potential. A root-to-shoot signal, particularly abscisic acid (ABA), promotes stomatal closure (Hasanuzzaman et al., 2020). Stomatal closure restricts carbon dioxide (CO_2) uptake and transpiration, thereby reducing the photosynthetic rate (Shalaby et al., 2021). The decline in photosynthesis is further aggravated by degradation of chlorophyll pigments and reduced photochemical efficiency of photosystem II (PSII; Fv/Fm) (Zahedi et al., 2020).

Drought disrupts the photosynthetic electron-transport chain and promotes excessive formation of reactive oxygen species (ROS; $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$) (Hasanuzzaman et al., 2020). ROS accumulation impairs cellular function through membrane lipid peroxidation, reflected by increased

malondialdehyde (MDA), oxidative damage to proteins, and deterioration of nucleic acids (Tsivileva, 2025). Lipid peroxidation compromises membrane integrity and contributes to ionic imbalance (Haghighi et al., 2014). Other important responses observed in drought-stressed vegetables include:

- Altered accumulation of osmotic regulators, particularly proline and soluble sugars;
- Impaired uptake of mineral nutrients, especially N, P, K, Ca, and Mg;
- Reductions in plant height, leaf area, and biomass; and
- Declines in yield and quality attributes, including fruit number, fruit weight, soluble solids (°Brix), and lycopene content (Zhu et al., 2018; Silva et al., 2020).

1.3. Physiological and Biochemical Effects of Salinity Stress on Vegetable Crops

Salinity is a major abiotic constraint that suppresses plant growth and development because of high concentrations of soluble salts in the soil solution (Munns and Tester, 2008). Salinity affects plants through three principal mechanisms:

1. Osmotic stress: The osmotic potential of the soil solution decreases, restricting plant water uptake. The resulting water deficit promotes stomatal closure, reduces photosynthesis, and slows growth (Munns and Tester, 2008).

2. Ion toxicity: Excessive accumulation of Na^+ and Cl^- in plant tissues disrupts cellular metabolism and inhibits enzyme activity (El-Ramady et al., 2020). Na^+ competes with K^+ for uptake and transport, thereby disturbing the Na^+/K^+ ratio (Hussain et al., 2020).

3. Nutrient imbalance: High Na^+ concentrations interfere with the uptake and translocation of essential nutrients such as Ca^{2+} and Mg^{2+}, resulting in chlorosis, necrosis, and growth suppression (Machado and Serralheiro, 2017).

Salinity also enhances ROS production, oxidative stress, and membrane lipid peroxidation, as indicated by increased MDA concentrations (Shalaby et al., 2021; Tsivileva, 2025). These processes increase membrane permeability and disrupt cellular functions (Fatima et al., 2024). Additional consequences in vegetable crops include:

- Inhibition of germination and seedling establishment (Behera et al., 2022);
- Slower vegetative and reproductive growth and reduced fruit set (Behera et al., 2022);

- Deterioration of fruit color, flavor, soluble-solids content, lycopene, and vitamin C; and
- Substantial yield losses (Zhu et al., 2018; Behera et al., 2022).

1.4. Antioxidant Defense and Oxidative-Stress Management in Plants

Plants possess a sophisticated antioxidant defense system that limits ROS accumulation under abiotic stress (Hasanuzzaman et al., 2020). This system comprises both enzymatic and non-enzymatic components.

Enzymatic antioxidants:

- **Superoxide dismutase (SOD):** converts $O_2^{\bullet -}$ radicals to H_2O_2.
- **Catalase (CAT):** decomposes H_2O_2 into water and oxygen.
- **Ascorbate peroxidase (APX):** reduces H_2O_2 to water within the ascorbate-glutathione cycle.
- **Glutathione reductase (GR):** regenerates reduced glutathione (GSH) from oxidized glutathione (GSSG).
- **Glutathione peroxidase (GPX):** detoxifies lipid hydroperoxides and H_2O_2.

Non-enzymatic antioxidants:

- Ascorbic acid (AsA);
- Glutathione (GSH);
- Phenolic compounds;
- Flavonoids;
- Carotenoids;
- Proline; and
- Soluble sugars.

These antioxidants protect cells by scavenging ROS (Tsivileva, 2025). When stress intensity exceeds the capacity of endogenous defense systems, however, oxidative damage becomes unavoidable and crop yield and quality decline (Hasanuzzaman et al., 2020). Exogenous treatments that reinforce antioxidant defense are therefore important tools for increasing stress tolerance.

1.5. The Role of Selenium in Plants: From a Beneficial Element to Stress Tolerance

Although selenium is not essential for higher plants, low Se doses are widely regarded as beneficial and may substantially enhance tolerance to

abiotic stress (El-Ramady et al., 2020; Hasanuzzaman et al., 2020). Selenium displays a biphasic, dose-dependent response known as hormesis: low doses are stimulatory and protective, whereas high doses are toxic and pro-oxidant (Fatima et al., 2024; Tsivileva, 2025). The principal mechanisms are as follows:

1. Strengthening antioxidant defense: Se participates in redox processes associated with glutathione peroxidase (GPX) and thioredoxin reductase (TrxR), and enhances ROS detoxification. Selenium treatments can activate the AsA-GSH cycle by increasing SOD, CAT, APX, and GR activities and can promote the accumulation of phenolics, flavonoids, and proline (Hasanuzzaman et al., 2020; Shalaby et al., 2021; Sardar et al., 2022; Tsivileva, 2025).

2. Protecting photosynthesis: Se limits chlorophyll loss, supports chlorophyll biosynthesis, improves PSII efficiency (Fv/Fm), and can enhance stomatal conductance (g_s) and net photosynthetic rate (A) under stress (Morales-Espinoza et al., 2019; Silva et al., 2020; Zahedi et al., 2020).

3. Promoting osmotic adjustment: Se supports the accumulation of proline, soluble sugars, and amino acids, helping cells maintain osmotic potential, water uptake, and turgor (Elkelish et al., 2019 Fatima et al., 2024; Tsivileva, 2025).

4. Regulating ion homeostasis: Under salinity stress, Se can decrease Na^+ uptake, increase K^+ acquisition, improve Na^+/K^+ and Na^+/Ca^{2+} ratios, and promote vacuolar sequestration of Na^+, thereby lowering cytosolic Na^+ concentrations (Hussain et al., 2020; Shriaiev et al., 2026).

1.6. The Emergence of Nano-Selenium (SeNPs): Why the Nanoscale?

Selenium nanoparticles have attracted considerable interest because they may offer advantages over ionic Se forms such as selenate and selenite (Fatima et al., 2024; Shriaiev et al., 2026). SeNPs are particles of elemental selenium (Se^0), generally within the size range of 2-282 nm, and can be synthesized by biological or chemical methods (Tsivileva, 2025). Their principal comparative properties are summarized below.

Property	Bulk Se (selenate/selenite)	Nano-selenium (SeNPs)
Bioavailability	Low to moderate	High
Toxicity	Higher; narrow safety range	Lower; wider safety range
Release profile	Rapid and immediate	Controlled and prolonged
Plant uptake	Dependent on ion transporters	Facilitated by nanoscale size
Antioxidant effect	Moderate	High
Environmental profile	Generally chemical synthesis	Biological synthesis is possible

The small size of SeNPs may facilitate passage through plant cell-wall pores, whereas their elemental Se^0 form generally confers lower toxicity than ionic forms (El-Ramady et al., 2020). Biogenic SeNPs synthesized with plant extracts or microorganisms possess organic surface coatings composed of proteins, lipids, and carbohydrates. These coatings can improve interactions with cellular membranes and thereby enhance particle uptake and bioavailability (El-Ramady et al., 2023; Tsivileva, 2025).

1.7. Aim and Scope of the Review

This review comprehensively evaluates Se and SeNP applications in vegetable crops subjected to drought and salinity stress, with emphasis on:

- Their physiological and biochemical mechanisms of action;
- Their effects on antioxidant defense, photosynthesis, osmotic adjustment, and ion homeostasis;
- Crop-specific responses in tomato, pepper, cucumber, lettuce, common bean, eggplant, broccoli, onion, and green bean;
- Optimum dose, application method, and timing; and
- Practical recommendations and future research priorities.

2. EFFECTS OF DROUGHT AND SALINITY STRESS ON VEGETABLE CROPS

Drought and salinity are two of the most important abiotic stresses causing yield and quality losses in vegetable crops (Munns and Tester, 2008; Farooq et al., 2009). Both stresses induce osmotic disturbance, ion imbalance, and oxidative injury, but differ in their primary causes and in some components of the plant response (Hasanuzzaman et al., 2020).

2.1. Drought Stress

2.1.1. Definition and Importance of Drought Stress

Drought develops when the water available in the soil is insufficient to meet crop demand and is among the most destructive abiotic stresses affecting plant growth, development, and productivity (Farooq et al., 2009). Climate change is increasing the frequency and intensity of drought and is particularly threatening the cultivation of water-sensitive vegetable species (IPCC, 2021). Drought risk across agricultural land therefore represents a major challenge to global food security (El-Ramady et al., 2023).

Vegetable crops are especially susceptible because of their high tissue water content and rapid growth. Drought may affect every developmental stage, from germination through harvest, and can substantially reduce both yield and market quality (Dias, 2019; Shalaby et al., 2021).

2.1.2. Physiological Effects of Drought Stress

The first major consequence of drought is a reduction in soil and plant water potential, which triggers several physiological responses (Farooq et al., 2009):

- **Stomatal closure and reduced gas exchange:** Root-derived ABA signals promote stomatal closure, restricting CO_2 uptake and transpiration. Consequently, photosynthesis, stomatal conductance (g_s), net assimilation (A), and frequently water-use efficiency (WUE) decline (Hasanuzzaman et al., 2020; Silva et al., 2020; Shalaby et al., 2021).
- **Photosynthetic inhibition and chlorophyll loss:** Drought reduces chlorophyll a and b, disturbs photosynthetic electron transport, and lowers PSII photochemical efficiency (Fv/Fm). Carotenoids may vary depending on stress severity and may also have a photoprotective function (Morales-Espinoza et al., 2019; Silva et al., 2020; Zahedi et al., 2020).
- **Osmotic stress and water loss:** Lower cellular osmotic potential causes water loss and reduced turgor, leading to loss of cell expansion, slower growth, and leaf wilting (Farooq et al., 2009; Haghighi et al., 2014).

2.1.3. Biochemical Effects of Drought Stress

Drought causes profound biochemical changes and oxidative injury in plants.

- **ROS production and oxidative stress:** Disturbance of photosynthetic and respiratory electron transport increases $O_2^{\bullet -}$, H_2O_2, and $OH^{\bullet}$ formation. Excess ROS promotes oxidative damage to membranes, proteins, and nucleic acids (Hasanuzzaman et al., 2020; Tsivileva, 2025).
- **Lipid peroxidation and membrane injury:** ROS attack unsaturated membrane fatty acids, increasing MDA and impairing membrane structure, ion selectivity, and cellular function (Shalaby et al., 2021; Fatima et al., 2024).
- **Changes in osmolyte accumulation:** Plants commonly accumulate proline, soluble sugars, and amino acids to lower cellular osmotic potential (Meena et al., 2019). Under severe stress, however, osmolyte synthesis may be insufficient to prevent dehydration (Blum, 2010).
- **Impaired mineral nutrition:** Reduced root water uptake limits the acquisition of N, P, K, Ca, and Mg, contributing to nutrient imbalance and growth suppression (Hussain et al., 2020; Behera et al., 2022; Shedeed et al., 2023).

2.1.4. Effects of Drought on Yield and Quality

Drought adversely affects major yield and quality traits in vegetable crops:

- **Fewer and lighter fruits:** Flower and fruit abscission reduce fruit number and individual fruit weight (Hernández-Hernández et al., 2019).
- **Lower fruit quality:** Soluble solids, titratable acidity, lycopene, vitamin C, and phenolic compounds may decline (Zhu et al., 2018).
- **Reduced marketability:** Color, flavor, texture, and nutritional value are often impaired under prolonged water deficit (Shriaiev et al., 2026).

2.1.5. Crop-Specific Effects of Drought Stress

- **Tomato (*Solanum lycopersicum*):** Drought reduces stomatal conductance and net photosynthesis, may caus %30-50 yield losses, and impairs soluble-solids and lycopene accumulation (Ishtiaq et al., 2023; Neysanian et al., 2024).

- **Pepper (*Capsicum annuum*):** Drought lowers WUE, fruit yield, phenolic compounds, and antioxidant activity (Gonzalez-Garcia et al., 2021; Sariñana-Navarrete et al., 2023).
- **Cucumber (*Cucumis sativus*):** Drought restricts plant growth, leaf area, chlorophyll content, and fruit yield (Shalaby et al., 2021).
- **Lettuce (*Lactuca sativa*):** Drought reduces relative water content and chlorophyll, alters phenolics and antioxidant capacity, and lowers leaf yield (Sardar et al., 2022).
- **Common bean (*Phaseolus vulgaris*):** Drought suppresses nodulation and biological nitrogen fixation, leading to reduced growth and yield (Hossain et al., 2022).

2.2. Salinity Stress

2.2.1. Definition and Importance of Salinity Stress

Salinity limits plant growth and development through high soluble-salt concentrations in the soil solution (Munns and Tester, 2008). Approximately one billion hectares of soil in the world are estimated to be affected by salinity (Shaygan and Baumgartl, 2022), with soil salinization continuing to expand, particularly in arid and semi-arid regions as a result of unsuitable irrigation practices and inadequate drainage (Daliakopoulos et al., 2021).

Vegetable crops range from moderately sensitive to highly sensitive to salinity (Dias, 2019). Salinity can impair germination, seedling establishment, vegetative growth, flowering, fruit set, and final yield (Shalaby et al., 2021).

2.2.2. Physiological Effects of Salinity Stress

The physiological impact of salinity is principally expressed through osmotic stress, ion toxicity, and nutrient imbalance (Munns and Tester, 2008).

- **Osmotic stress:** A lower soil-solution osmotic potential restricts water uptake and causes stomatal closure, reduced photosynthesis, lower relative water content (RWC), loss of turgor, and slower metabolism and growth (Munns and Tester, 2008; Farooq et al., 2009; Haghighi et al., 2014).
- **Ion toxicity:** Na^+ and Cl^- accumulate in tissues and damage membranes and metabolic enzymes. Na^+ also competes with K^+, impairing K^+ uptake and disturbing the Na^+/ K^+ ratio (El-Ramady et al., 2020; Hussain et al., 2020; Behera et al., 2022).
- **Nutrient imbalance:** High Na^+ concentrations restrict uptake and transport of K^+, Ca^{2+}, and Mg^{2+}, leading to deficiency symptoms such

as chlorosis, necrosis, and growth retardation (Behera et al., 2022; Shedeed et al., 2023).

2.2.3. Biochemical Effects of Salinity Stress

Salinity induces substantial biochemical disruption and oxidative injury.

- **ROS production and oxidative stress:** Disruption of photosynthetic and respiratory electron transport results in excessive ROS formation and damage to membrane lipids, proteins, and DNA (Fatima et al., 2024; Tsivileva, 2025).
- **Membrane damage and lipid peroxidation:** Peroxidation of unsaturated fatty acids increases MDA, compromises membrane integrity, and increases ion leakage (Shalaby et al., 2021; Fatima et al., 2024).
- **Osmolyte accumulation:** Plants accumulate proline, glycine betaine, soluble sugars, and amino acids to improve osmotic adjustment, although this response may be inadequate under severe salinity (Elkelish et al., 2019; Sardar et al., 2022).
- **Activation of antioxidant defense:** Activities of SOD, CAT, APX, and GR commonly increase, but oxidative injury occurs when stress intensity exceeds antioxidant capacity (Hasanuzzaman et al., 2020; Tsivileva, 2025).

2.2.4. Effects of Salinity on Yield and Quality

Salinity substantially reduces yield and quality in vegetable crops:

- **Yield loss:** Growth inhibition, flower and fruit abscission, and reduced fruit number and weight decrease total and marketable yield (Behera et al., 2022; Sariñana-Navarrete et al., 2023).
- **Lower fruit quality:** Soluble solids, titratable acidity, lycopene, vitamin C, and phenolics may decline (Zhu et al., 2018).
- **Reduced nutritional value:** Concentrations of K, Ca, and Mg may be adversely affected (Shedeed et al., 2023).

2.2.5. Crop-Specific Effects of Salinity Stress

- **Tomato (*Solanum lycopersicum*):** Salinity reduces fruit weight, fruit number, soluble solids, lycopene, and vitamin C. Na^+ toxicity and disruption of the Na^+ /K^+ ratio are prominent constraints (Zhu et al., 2018; Morales-Espinoza et al., 2019; Behera et al., 2022).

- **Pepper (*Capsicum annuum*):** Salinity reduces fruit yield and quality and may lower phenolic compounds and antioxidant activity (Gonzalez-Garcia et al., 2021; Sariñana-Navarrete et al., 2023).
- **Cucumber (*Cucumis sativus*):** Salinity restricts growth, chlorophyll concentration, and fruit yield (Shalaby et al., 2021).
- **Lettuce (*Lactuca sativa*):** Salinity reduces leaf yield, mineral nutrition, and antioxidant capacity (Hawrylak-Nowak, 2015; Sardar et al., 2022).
- **Common bean (*Phaseolus vulgaris*):** Salinity impairs nodulation and nitrogen fixation and thereby reduces yield (Hossain et al., 2022).

2.3. Comparative Evaluation of Drought and Salinity Stress

Drought and salinity induce several common physiological and biochemical disturbances, including osmotic stress, oxidative injury, and nutrient imbalance, but differ in their initiating factors and ion-specific effects (Table 1).

Table 1. Comparative effects of drought and salinity stress on selected vegetable crops

Characteristic	Drought stress	Salinity stress
Primary cause	Decline in soil water potential	High salt concentration in the soil solution
Initial effect	Water deficit and dehydration	Osmotic stress and ion toxicity
Ion toxicity	Generally absent	Na+ and K+ toxicity
Stomatal response	ABA-mediated closure	ABA- and ion-mediated closure
Nutrient imbalance	Reduced acquisition	Reduced acquisition plus Na^+/K^+ competition
Root response	May increase root/shoot ratio in search of water	Root growth is commonly suppressed
Osmotic adjustment	Proline and sugar accumulation	Proline, glycine betaine, and sugar accumulation
Antioxidant response	Increased SOD, CAT, and APX	Increased SOD, CAT, and APX with distinct regulation
Yield loss	High; often 30-50%	High; often %30-70, depending on crop
Quality loss	Lower soluble solids, lycopene, and vitamin C	Similar quality losses plus Na^+ accumulation
Sensitive crops	Tomato, lettuce, common bean	Tomato, pepper, lettuce, common bean
Relatively tolerant crops	Pepper and eggplant, partially	Cucumber and eggplant, partially

Source: Compiled from Munns and Tester (2008), Farooq et al. (2009), Hasanuzzaman et al. (2020), Shalaby et al. (2021), Behera et al., 2022, and Shedeed et al. (2023).

Table 1 note: Both stresses cause osmotic and oxidative injury. The defining additional features of salinity are Na^+ and Cl^- toxicity and nutrient imbalance driven partly by Na^+/K^+ competition. These distinctions are central to the design of crop-specific stress-management strategies.

2.4. Summary of Drought and Salinity-Induced Damage in Vegetable Crops

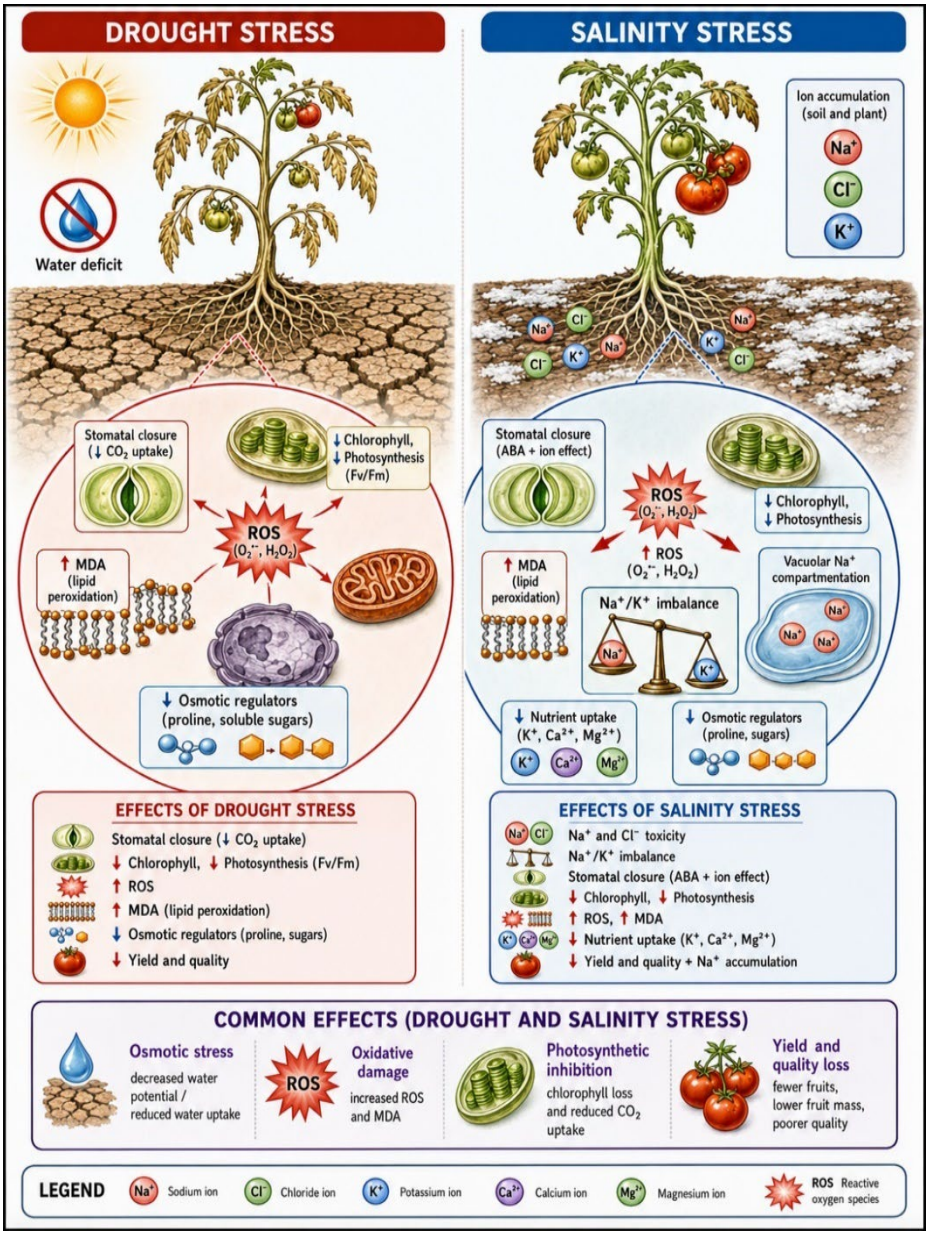

Figure 1. Major physiological and biochemical damage caused by drought and salinity stress in vegetable crops.

3. THE ROLE OF SELENIUM IN PLANTS

Although selenium is not essential for higher plants, it is considered a beneficial element at low doses and can play an important role in enhancing tolerance to abiotic stress (El-Ramady et al., 2020; Hasanuzzaman et al., 2020). Its effect is dose dependent and follows a hormetic pattern: low concentrations are stimulatory and protective, whereas excessive concentrations are toxic and pro-oxidant (Fatima et al., 2024; Tsivileva, 2025). This section details the principal mechanisms through which Se influences plant stress responses.

3.1. Selenium Uptake and Metabolism in Plants

Plants acquire selenium from the soil primarily as selenate and selenite through transport systems shared with chemically analogous nutrients, particularly sulfate and phosphate (Schiavon and Pilon-Smits, 2017). Selenate (SeO_4^{2-}) competes with sulfate for uptake by root sulfate transporters and is readily transported to leaves through the xylem (El-Ramady et al., 2020). Selenite (SeO_3^{2-}) is taken up through phosphate transporters and passive pathways and is generally less mobile within the plant (Hasanuzzaman et al., 2020). Selenium metabolism broadly parallels sulfur metabolism:

1. **Selenate → selenite:** Selenate is activated and reduced through reactions involving ATP sulfurylase (Schiavon and Pilon-Smits, 2017).
2. **Selenite → selenide:** Selenite is reduced to selenide (Se^{2-}) through reactions involving glutathione and other redox-active compounds (El-Ramady et al., 2020).
3. **Selenide → selenoamino acids:** Selenide reacts with O-acetylserine to form selenocysteine (SeCys) and is subsequently incorporated into selenomethionine (SeMet) and related compounds (Hasanuzzaman et al., 2020).

At high concentrations, selenoamino acids may be non-specifically incorporated into proteins and cause toxicity (Tsivileva, 2025). At suitable low doses, however, Se-containing compounds contribute to redox regulation and antioxidant defense.

3.2. Enhancement of the Antioxidant System by Selenium

One of the most important roles attributed to Se in plants is reinforcement of antioxidant defense through both enzymatic and non-enzymatic mechanisms (Hasanuzzaman et al., 2020).

3.2.1. Activation of Selenium-Associated Redox Enzymes

Se is associated with redox processes involving glutathione peroxidase (GPX) and thioredoxin reductase (TrxR), which contribute to ROS detoxification and maintenance of cellular redox balance (Hasanuzzaman et al., 2020). GPX reduces lipid hydroperoxides and H_2O_2, whereas TrxR regenerates reduced thioredoxin and supports redox homeostasis (Tsivileva, 2025).

3.2.2. Activation of the Ascorbate-Glutathione Cycle

Selenium application may increase the activities of enzymes in the ascorbate-glutathione (AsA-GSH) cycle (Shalaby et al., 2021). This cycle includes APX, GR, dehydroascorbate reductase (DHAR), and monodehydroascorbate reductase (MDHAR) (Hasanuzzaman et al., 2020). By stimulating these enzymes, Se supports the regeneration of ascorbate and glutathione and improves ROS scavenging (Tsivileva, 2025).

3.2.3. Activation of Other Antioxidant Enzymes

Selenium treatments can also enhance SOD, CAT, and peroxidase (POD) activities (Fatima et al., 2024; Shriaiev et al., 2026). SOD converts O_2^- into H_2O_2, while CAT and POD subsequently convert H_2O_2 into less harmful products (Haghighi et al., 2014).

3.2.4. Promotion of Non-Enzymatic Antioxidants

Se can stimulate the accumulation of phenolics, flavonoids, carotenoids, and proline (Sardar et al., 2022). These metabolites contribute directly or indirectly to ROS scavenging and reduce oxidative injury (El-Ramady et al., 2020).

3.3. The Role of Selenium in Protecting Photosynthesis

Selenium contributes to the maintenance of photosynthetic function under abiotic stress (Morales-Espinoza et al., 2019; Zahedi et al., 2020).

3.3.1. Chlorophyll Protection and Biosynthesis

Se application may prevent or reduce stress-induced chlorophyll loss (Haghighi et al., 2014). By supporting pigment biosynthesis and limiting chlorophyll degradation, Se helps maintain photosynthetic capacity (Silva et al., 2020). In tomato, Se treatments have significantly increased chlorophyll a and b concentrations under stress (Morales-Espinoza et al., 2019).

3.3.2. Improvement of PSII Efficiency

Selenium can increase the photochemical efficiency of PSII, commonly assessed as Fv/Fm, and thereby improve the use of absorbed light energy under stress (Zahedi et al., 2020; Shriaiev et al., 2026).

3.3.3. Improvement of Gas Exchange

At appropriate concentrations, Se application may alleviate stress-induced reductions in photosynthetic gas exchange. Soil-applied Se improved CO_2 assimilation and stomatal conductance in drought-stressed rice, whereas 0.01 mM Na_2SeO_3 partially restored net photosynthetic rate and stomatal conductance in salt-stressed tomato seedlings (Andrade et al., 2018; Zhang et al., 2023).

3.4. The Role of Selenium in Osmotic Adjustment and Ion Homeostasis

Selenium contributes to stress tolerance by supporting osmotic adjustment and regulating ion homeostasis (Hussain et al., 2020; Tsivileva, 2025).

3.4.1. Promotion of Osmolyte Accumulation

Se can stimulate the accumulation of proline, soluble sugars, and amino acids (Hasanuzzaman et al., 2020). These compounds lower cellular osmotic potential, facilitate water uptake, preserve turgor, and improve tissue water status (Fatima et al., 2024). Proline accumulation is particularly important under drought because it contributes to water retention and protection against dehydration (Sardar et al., 2022).

3.4.2. Regulation of Ion Balance

Under salinity stress, Se can reduce Na^+ uptake and increase K^+ uptake, improving Na^+/K^+ and Na^+/Ca^{2+} ratios (Hussain et al., 2020). It may also

promote vacuolar compartmentation of Na^+, lower cytosolic Na^+ concentrations, and reduce ion toxicity (Shriaiev et al., 2026). Improved acquisition of K^+, Ca^2, and Mg^{2+} may further correct salt-induced nutrient imbalance (Yaldız and Çamlıca, 2022).

3.5. Selenium Toxicity and Pro-Oxidant Effects: Hormesis

The response of plants to selenium is strongly dose dependent and follows a hormetic pattern (Fatima et al., 2024; Tsivileva, 2025).

3.5.1. Low Dose: Beneficial Effects

At low concentrations, frequently below approximately 50 mg L^{-1} or 100 μM depending on crop and formulation, Se may:

- strengthen antioxidant defense;
- protect photosynthesis;
- improve osmotic adjustment;
- increase stress tolerance; and
- promote growth and yield (Hasanuzzaman et al., 2020).

3.5.2. High Dose: Toxic Effects

At excessive concentrations, frequently above approximately 50 mg L^{-1} or 100 μM depending on crop and formulation, Se may:

- increase ROS production and exert a pro-oxidant effect;
- promote lipid peroxidation;
- cause chlorophyll loss;
- inhibit growth; and
- increase non-specific incorporation of selenoamino acids into proteins (Tsivileva, 2025).

Visible symptoms of Se toxicity include chlorosis, necrosis, growth retardation, and yield loss (Fatima et al., 2024). Successful Se use therefore depends on careful dose optimization (Table 2).

Table 2. Dose-dependent effects of selenium in plants (hormesis)

Dose	Response	Principal mechanisms	Expected outcome
Low (<10 mg L^{-1})	Beneficial / stimulatory	• GPX and TrxR-associated redox activity • Activation of the AsA-GSH cycle • Increased SOD, CAT, and APX • Promotion of proline and sugars	• Stronger antioxidant defense • Protected photosynthesis • Greater stress tolerance • Improved yield and quality
Moderate (10-50 mg L^{-1})	Optimal	• Balanced antioxidant activation • Controlled formation of Se metabolites	• Maximum benefit • No evident toxicity
High (>50 mg L^{-1})	Toxic / pro-oxidant	• Excess ROS generation • Misincorporation of selenoamino acids • Inhibition of antioxidant enzymes	• Increased MDA and lipid peroxidation • Chlorophyll loss and growth inhibition • Reduced yield and quality • Chlorosis and necrosis

Source: Compiled from El-Ramady et al. (2020), Hasanuzzaman et al. (2020), Fatima et al. (2024), and Tsivileva (2025).

Table 2 note: Low Se doses may strengthen antioxidant defense and protect photosynthesis, whereas excessive doses become pro-oxidant and phytotoxic. The actual optimum depends on crop species, growth stage, Se formulation, application route, and environmental conditions.

3.6. Summary of Selenium-Mediated Stress-Tolerance Mechanisms

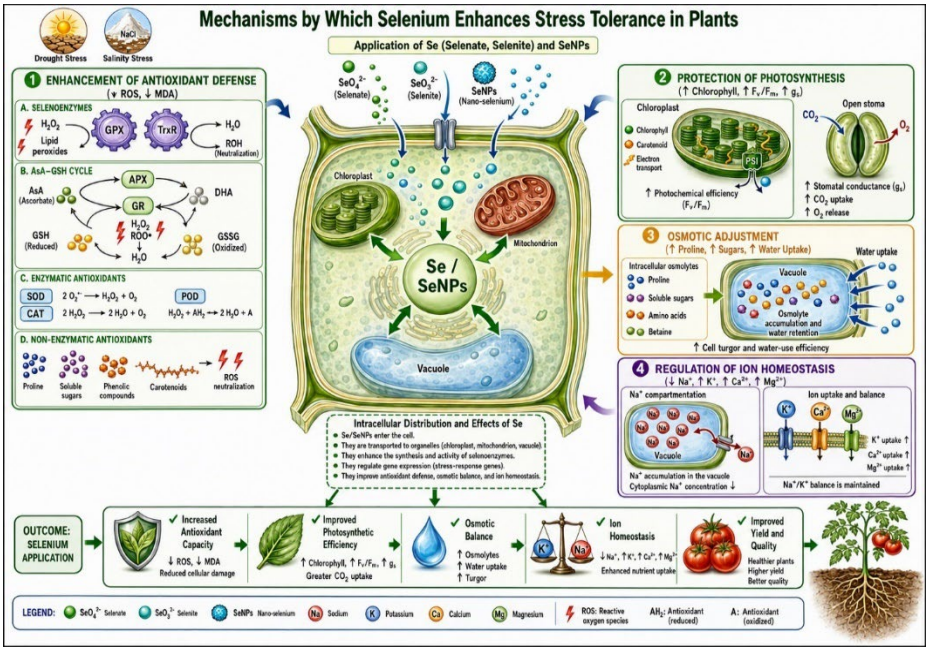

Figure 2. Principal mechanisms through which selenium and nano-selenium enhance plant tolerance to abiotic stress.

4. ADVANTAGES OF NANO-SELENIUM (SeNPs)

Nanotechnology provides new opportunities for sustainable crop production and food security (Gaikwad et al., 2024). Nano-selenium consists of elemental Se^0 particles, generally 2-282 nm in size, synthesized through physical, chemical, or biological routes (Tsivileva, 2025; Shriaiev et al., 2026). Compared with ionic Se forms, SeNPs may provide higher bioavailability, controlled release, and a wider operational safety range (Fatima et al., 2024).

4.1. Synthesis Methods for Nano-Selenium

SeNPs can be synthesized by three broad approaches: physical, chemical, and biological or biogenic methods (Fatima et al., 2024).

4.1.1. Physical Methods

- **Laser ablation:** high-energy laser irradiation removes particles from a selenium target.
- **Evaporation-condensation:** selenium is vaporized at high temperature and then rapidly cooled.
- **Mechanical milling:** bulk selenium is ground to the nanoscale.

The major advantage of physical synthesis is the production of relatively pure particles, whereas major limitations include high energy consumption and low throughput.

4.1.2. Chemical Methods

- **Chemical reduction:** selenite or selenate salts are reduced to elemental Se^0 using agents such as ascorbic acid, sodium borohydride, or glycols (Fatima et al., 2024).
- **Sol-gel synthesis:** selenium precursors undergo hydrolysis and condensation reactions.
- **Microemulsion synthesis:** nanoparticles are formed in nanoscale reaction compartments.

Chemical methods permit relatively precise control of particle size and shape but may require toxic reagents and incur high production costs.

4.1.3. Biological (Biogenic) Methods

Biogenic SeNPs are synthesized using plant extracts, bacteria, fungi, or algae (El-Ramady et al., 2023; Tsivileva, 2025). These approaches are

generally considered more environmentally compatible, comparatively low cost, and biocompatible (Fatima et al., 2024). Examples include:

- **Plant extracts:** extracts from *Vitis vinifera, Aloe vera*, and *Azadirachta indica* (Sharma et al., 2014; Fardsadegh and Jafarzadeh-Malmiri, 2019; Mulla et al., 2020).
- **Bacteria:** *Bacillus subtilis* has been extensively investigated as a microbial system for selenium nanoparticle biosynthesis (Ullah et al., 2021).
- **Fungi:** *Aspergillus terreus* and *Fusarium oxysporum* (Zare et al., 2013; Islam et al., 2022).
- **Algae:** *Spirulina platensis* and *Ulva fasciata* (Alipour et al., 2021.

The organic coating formed during biological synthesis may contain proteins, lipids, and carbohydrates and can improve particle stability and bioavailability (Tsivileva, 2025).

4.2. Advantages of Nano-Selenium over Ionic Selenium

The principal differences between ionic Se and SeNPs are summarized in Table 3.

Table 3. Comparison of nano-selenium and ionic selenium (selenate/selenite)

Property	Ionic selenium (selenate/selenite)	Nano-selenium (SeNPs)
Chemical form	SeO_4^{2-} and SeO_3^{2-}	Elemental Se^0
Particle state	Dissolved ions	Generally, 2-282 nm particles
Bioavailability	Low to moderate	High
Plant uptake	Dependent on sulfate/phosphate transporters	Root and foliar entry; nanoscale penetration may contribute
Toxicity	Higher; narrower safety range	Lower acute toxicity; wider operating range
Release profile	Rapid	Controlled and prolonged
Antioxidant response	Moderate	Often stronger
Environmental profile	Commonly produced chemically	Biogenic synthesis is possible
Stability	Stable in solution as ions	Coating may be required; aggregation risk
Cost	Generally lower	Moderate to high, depending on synthesis
Biofortification potential	Moderate	High, with conversion to organic Se forms

Source: Compiled from El-Ramady et al. (2020), Fatima et al. (2024), Tsivileva (2025), and Shriaiev et al. (2026).

Table 3 note: SeNPs may provide higher bioavailability, lower acute toxicity, controlled release, and stronger antioxidant responses. Biogenic SeNPs are especially attractive because their synthesis can be environmentally compatible and their organic coatings may enhance particle stability and plant interaction (El-Ramady et al., 2023).

4.2.1. Higher Bioavailability and Uptake

The nanoscale size and physicochemical properties of SeNPs facilitate their interaction with plant tissues and enhance their uptake and translocation within plants (Avellan et al., 2021; Fatima et al., 2024). Nanoparticles may enter through root hairs, root tips, stomata, and the leaf cuticle and subsequently move through xylem and phloem pathways (Shriaiev et al., 2026). Organic coatings on biogenic SeNPs may further improve membrane interaction and uptake (Tsivileva, 2025).

4.2.2. Lower Toxicity and a Wider Safety Range

A key potential advantage of SeNPs is lower acute phytotoxicity than ionic selenate or selenite (Fatima et al., 2024; Shriaiev et al., 2026). Ionic forms may cause chlorosis, necrosis, and growth inhibition at high concentrations, whereas the elemental Se^0 form and slower release of SeNPs can reduce rapid exposure and toxicity risk (El-Ramady et al., 2020; Tsivileva, 2025). This may allow SeNPs to be used over a broader dose range, although formulation-specific testing remains essential.

4.2.3. Controlled Release and Prolonged Action

SeNPs can act as a more gradual source of selenium than rapidly absorbed ionic forms (Fatima et al., 2024). Controlled release may provide a more sustained supply, which is particularly valuable during prolonged stress episodes (El-Ramady et al., 2020).

4.2.4. Stronger Antioxidant and Stress-Mitigation Effects

SeNPs often elicit strong activation of SOD, CAT, APX, and GPX and thereby enhance ROS detoxification (Fatima et al., 2024; Tsivileva, 2025; Shriaiev et al., 2026). They may also increase phenolics, flavonoids, and proline. These responses have been associated with improved tolerance to drought, salinity, temperature extremes, and heavy-metal stress (Sardar et al., 2022).

4.2.5. Biofortification and Potential Benefits for Human Nutrition

SeNPs can increase the selenium concentration of edible plant tissues and therefore represent a potentially effective biofortification tool (El-Ramady

et al., 2023; Shriaiev et al., 2026). Application may promote accumulation of organic Se forms, including selenomethionine and selenocysteine, which are generally more bioavailable to humans than inorganic forms (El-Ramady et al., 2020; Tsivileva, 2025). In this respect, SeNP-based biofortification may simultaneously support crop stress tolerance and improve the nutritional value of harvested produce.

4.3. Uptake and Transport of Nano-Selenium in Plants

SeNPs can be absorbed by both roots and leaves (Fatima et al., 2024).

4.3.1. Root Uptake and Transport

Root-supplied SeNPs can be absorbed and biotransformed by vegetable crops, but their root-to-shoot mobility is generally lower than that of selenate. Available studies indicate substantial root retention and conversion of SeNP-derived Se into organic Se compounds, whereas efficient xylem transport of intact SeNPs has not been conclusively demonstrated, including in vegetable crops subjected to salinity stress (Morales-Espinoza et al., 2019; Li et al., 2020; Moreno-Martín et al., 2020;).

4.3.2. Foliar Uptake and Transport

Following foliar application, SeNPs may enter through stomata and the cuticle (Avellan et al., 2021). They can then move from cell to cell through the apoplast and plasmodesmata or be redistributed through the phloem (Fatima et al., 2024). Foliar application generally produces a faster and more targeted response than root-zone application (Shriaiev et al., 2026).

4.4. Mechanisms of Nano-Selenium-Mediated Stress Tolerance

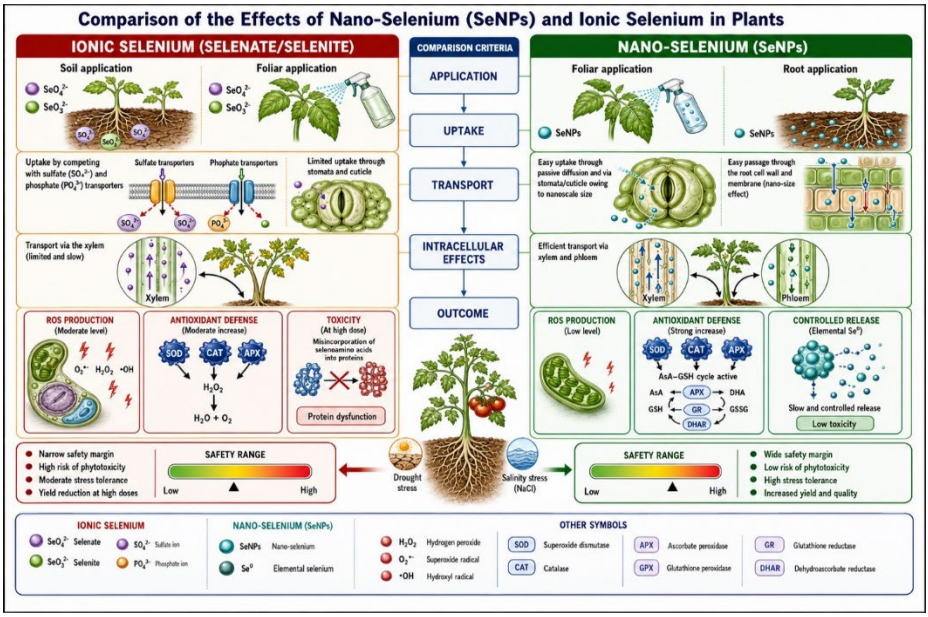

Figure 3. Comparative uptake, transport, biological effects, and practical characteristics of ionic selenium and nano-selenium in plants.

5. STUDIES CONDUCTED IN VEGETABLE CROPS

The effects of Se and SeNP applications under drought and salinity vary with crop species, Se formulation, dose, and application method (Fatima et al., 2024; Shriaiev et al., 2026). This section summarizes representative findings for tomato, pepper, cucumber, lettuce, common bean, eggplant, broccoli, onion, and green bean, including major physiological, biochemical, yield, and quality responses (Table 4).

5.1. Tomato (*Solanum lycopersicum*)

Tomato (*Solanum lycopersicum* L.) is one of the most widely cultivated vegetable crops and a model dicot plant. It is moderately sensitive to salinity throughout the period of growth and development (Guo et al., 2022). Consequently, Se and SeNP treatments have been studied extensively in tomato (Hernández-Hernández et al., 2019; Neysanian et al., 2020).

5.1.1. Se and SeNP Applications in Tomato under Salinity Stress

NaCl stress adversely affects tomato fruit yield, quality, and nutritional composition (Zhu et al., 2018).

Morales-Espinoza et al. (2019) found that 20 mg L^{-1} SeNPs supplied to tomato plants exposed to 50 mM NaCl increased chlorophyll a and b by %71 and %120, respectively, increased individual fruit weight by %13, and improved titratable acidity. SeNP treatment also increased APX, CAT, and SOD activities and lowered MDA.

El-Badri et al. (2022) reported that biogenic SeNPs at 5 mg L^{-1} enhanced SOD, CAT, and GPX activities, reduced H_2O_2 and MDA, and increased proline and soluble sugars under 150 mM NaCl. The study indicated that biogenic SeNPs could be more effective than chemically synthesized SeNPs under the evaluated conditions.

5.1.2. Se and SeNP Applications in Tomato under Drought Stress

Neysanian et al. (2024) reported that foliar application of 10-20 mg L^{-1} SeNPs to drought-stressed tomato increased chlorophyll, Fv/Fm, proline, and soluble sugars; reduced MDA and H_2O_2; and increased SOD, CAT, and POD activities.

Ishtiaq et al. (2023) found that seed priming with 75 ppm SeNPs reduced oxidative stress, enhanced antioxidant enzyme activity, and improved phytonutrient accumulation in water-stressed tomato plants.

5.1.3. Practical Application Guidance for Tomato

- **Target stress:** salinity and drought.
- **Application stage:** seedling stage at 3-4 true leaves and the onset of flowering.
- **Preferred method:** foliar spray.
- **Suggested SeNP range:** 10-20 mg L^{-1}.
- **Suggested ionic Se range:** 0.5-1 mM Na_2SeO_4.
- **Frequency:** 2-4 applications at intervals of 7-15 days.
- **Alternative seed priming:** 75 ppm SeNPs for 6-8 h.
- **Expected response:** yield increases of up to approximately %21, higher soluble solids and lycopene, and reduced salt injury.

- **Caution:** crop response must be verified locally because some tomato genotypes may exhibit phytotoxicity at SeNP concentrations above 10 mg L^{-1}.

5.2. Pepper (*Capsicum annuum*)

Pepper is an economically important vegetable crop with moderate sensitivity to salinity (Gonzalez-Garcia et al., 2021; Sariñana-Navarrete et al., 2023).

5.2.1. Se and SeNP Applications in Pepper under Salinity Stress

Gonzalez-Garcia et al. (2021) showed that SeNP application to pepper plants subjected to 25-50 mM NaCl increased leaf phenolics, flavonoids, GSH, and β-carotene and enhanced APX, GPX, CAT, and phenylalanine ammonia-lyase (PAL) activities.

Sariñana-Navarrete et al. (2023) reported that root-zone application of 45 mg L^{-1} SeNPs increased yield by %52.75 and enhanced vitamin C, total phenolics, and peroxidase activity in salt-stressed pepper.

Kanber et al. (2025) applied 5-20 mg L^{-1} biogenic SeNPs synthesized with *Sternbergia candida* to pepper plants under 100 mM NaCl. Foliar treatment reduced proline and MDA, increased chlorophyll, carotenoids, phenolics, and antioxidant activity, and produced the strongest response at 20 mg L^{-1}.

Under nutrient-deficient sandy-soil conditions, Shedeed et al. (2023) reported that 20-30 ppm SeNPs or 20 ppm conventional Se improved growth, plant nutritional status, and yield.

5.2.2. Practical Application Guidance for Pepper

- **Target stress:** salinity and nutrient deficiency.
- **Application stage:** vegetative growth and fruit set.
- **Preferred method:** foliar spray or root-zone application.
- **Suggested SeNP range:** 20-30 mg L^{-1} as a foliar spray or 20-30 ppm through the root zone.
- **Suggested ionic Se rate:** 20 ppm Na_2SeO_3.
- **Frequency:** 2-4 applications at intervals of 10-15 days.

- **Expected response:** yield increases approaching 53%, higher phenolic and antioxidant activity, and increased vitamin C.
- **Caution:** toxicity risk increases at rates above approximately 45 mg L^{-1}.

5.3. Cucumber (*Cucumis sativus*)

Cucumber is sensitive to salinity and high-temperature stress (Shalaby et al., 2021).

5.3.1. Se and SeNP Applications in Cucumber under Combined Salinity and Heat Stress

Shalaby et al. (2021) reported that foliar application of 25 mg L^{-1} SeNPs to cucumber exposed to 75 mM NaCl and high temperature increased plant height and leaf area, maintained chlorophyll, enhanced APX, CAT, and SOD activities, and lowered MDA. Application of 0.5-1 mg L^{-1} Na_2SeO_4 produced broadly similar responses.

5.3.2. Practical Application Guidance for Cucumber

- **Target stress:** salinity and heat stress.
- **Application stage:** 4-5 true-leaf stage.
- **Preferred method:** foliar spray.
- **Suggested SeNP rate:** 25 mg L^{-1}.
- **Suggested ionic Se range:** 0.5-1 mg L^{-1} Na_2SeO_4.
- **Frequency:** three applications at two-week intervals.
- **Expected response:** greater plant height and leaf area, higher antioxidant enzyme activity, and lower MDA and H_2O_2.
- **Interpretive note:** benefits may be more evident when salinity and heat occur simultaneously.

5.4. Lettuce (*Lactuca sativa*)

Lettuce is a leafy vegetable that is sensitive to drought and salinity stress (Hawrylak-Nowak, 2015; Sardar et al., 2022).

5.4.1. Se and SeNP Applications in Lettuce under Drought and Salinity Stress

Sardar et al. (2022) reported that foliar application of 5-10 µM Na_2SeO_4 to drought-stressed lettuce maintained relative water content, increased phenolics and antioxidant capacity measured by DPPH and FRAP assays,

promoted proline and soluble-sugar accumulation, lowered MDA, and improved WUE.

Hawrylak-Nowak (2015) found that 2-30 µM selenate produced a hormetic response in salt-stressed lettuce: 2-4 µM promoted growth, whereas 15-30 µM caused phytotoxicity.

5.4.2. Practical Application Guidance for Lettuce

- **Target stress:** drought and salinity.
- **Application stage:** seedling stage at 3-4 true leaves.
- **Preferred method:** foliar application.
- **Suggested ionic Se range:** 5-10 µM Na_2SeO_4.
- **Suggested SeNP range:** 5-10 mg L^{-1}.
- **Frequency:** one or two applications.
- **Expected response:** maintenance of RWC, higher phenolics and antioxidant capacity, and improved WUE.
- **Caution:** phytotoxicity becomes increasingly likely above approximately 15 µM ionic Se.

5.5. Common Bean (*Phaseolus vulgaris*)

Common bean is moderately tolerant to salinity relative to highly sensitive vegetables and is particularly important because salinity can impair nodulation and biological nitrogen fixation (Hossain et al., 2022; Shedeed et al., 2023).

5.5.1. Se and SeNP Applications in Common Bean under Salinity Stress

Hossain et al. (2022) reported that Na_2SeO_4 application promoted nodulation and nitrogen fixation, enhanced antioxidant enzyme activity, and improved salinity tolerance in common bean. The crop was reported to tolerate Se better than tomato under the compared conditions.

In nutrient-deficient sandy soil, Shedeed et al. (2023) found that 20-30 ppm SeNPs or 20 ppm conventional Se improved growth, nutritional status, and yield.

5.5.2. Practical Application Guidance for Common Bean

- **Target stress:** salinity and nutrient deficiency.
- **Application stage:** vegetative growth.

- **Preferred method:** foliar application.
- **Suggested SeNP range:** 20-30 ppm.
- **Suggested ionic Se rate:** 20 ppm Na_2SeO_3.
- **Frequency:** two applications.
- **Expected response:** improved nodulation, nitrogen fixation, growth, nutrient status, and salinity tolerance.
- **Interpretive note:** common bean may tolerate Se better than tomato, but genotype-specific testing remains necessary.

5.6. Eggplant (*Solanum melongena*)

Eggplant is responsive to mineral-nutrition constraints, particularly under low-fertility sandy-soil conditions (El-Ramady et al., 2023; Mahmoud et al., 2023).

5.6.1. Se and SeNP Applications in Eggplant under Nutrient-Deficiency Stress

Mahmoud et al. (2023) reported that 30-40 ppm SeNPs or conventional Se improved seedling growth, increased yield, and enhanced mineral nutrient uptake in eggplant grown under nutrient-deficient sandy-soil conditions.

5.6.2. Practical Application Guidance for Eggplant

- **Target stress:** nutrient deficiency in sandy soil.
- **Application stage:** seedling and vegetative growth stages.
- **Preferred method:** foliar application.
- **Suggested SeNP range:** 30-40 ppm.
- **Suggested ionic Se range:** 30-40 ppm Na_2SeO_3.
- **Frequency:** two applications, approximately 45 and 55 days after establishment.
- **Expected response:** improved seedling growth, yield, and mineral nutrient acquisition.

5.7. Broccoli (*Brassica oleracea var. italica*)

Broccoli is a high-value vegetable crop that may respond strongly to mineral-nutrient limitations in sandy soils (Abdalla et al., 2023; El-Ramady et al., 2023).

5.7.1. Se and SeNP Applications in Broccoli under Nutrient-Deficiency Stress

Abdalla et al. (2023) reported that 30-40 ppm SeNPs or conventional Se increased head weight and total yield and enhanced glucosinolate and phenolic concentrations in broccoli grown under nutrient-deficiency stress.

5.7.2. Practical Application Guidance for Broccoli

- **Target stress:** nutrient deficiency in sandy soil.
- **Application stage:** vegetative growth.
- **Preferred method:** foliar application.
- **Suggested ionic Se range:** 30-40 ppm Na_2SeO_3.
- **Suggested SeNP range:** 30-40 ppm.
- **Frequency:** two applications.
- **Expected response:** higher head weight and yield and increased glucosinolates and phenolic compounds.

5.8. Onion (*Allium cepa*)

Onion is an economically important vegetable that may exhibit strong responses to nutrient deficiency in sandy soils (El-Bassiony et al., 2023; El-Ramady et al., 2023).

5.8.1. Se and SeNP Applications in Onion under Nutrient-Deficiency Stress

El-Bassiony et al. (2023) reported that 30-40 ppm SeNPs or conventional Se increased bulb weight and yield and enhanced N, P, and K concentrations in onion under nutrient-deficient sandy-soil conditions.

5.8.2. Practical Application Guidance for Onion

- **Target stress:** nutrient deficiency in sandy soil.
- **Application stage:** vegetative growth.
- **Preferred method:** foliar application.
- **Suggested ionic Se range:** 30-40 ppm Na_2SeO_3.
- **Suggested SeNP range:** 30-40 ppm.
- **Expected response:** higher bulb weight and yield and improved N, P, and K status.

5.9. Green Bean (*Phaseolus vulgaris*)

Green bean is widely consumed as a fresh vegetable and may respond markedly to low nutrient availability in sandy soils (Shedeed et al., 2023).

5.9.1. Se and SeNP Applications in Green Bean under Nutrient-Deficiency Stress

Shedeed et al. (2023) reported that 20-30 ppm SeNPs or 20 ppm conventional Se increased fresh-pod yield, improved pod quality, and enhanced mineral nutrient content under nutrient-deficiency stress.

5.9.2. Practical Application Guidance for Green Bean

- **Target stress:** nutrient deficiency in sandy soil.
- **Application stage:** vegetative growth.
- **Preferred method:** foliar application.
- **Suggested SeNP range:** 20-30 ppm.
- **Suggested ionic Se rate:** 20 ppm Na_2SeO_3.
- **Expected response:** higher fresh-pod yield, improved pod quality, and increased mineral nutrient content.

5.10. Comparative Summary of Se and SeNP Applications in Vegetable Crops

Table 4. Effects of selenium and nano-selenium on physiological, biochemical, yield, and quality traits in vegetable crops exposed to abiotic stress

Crop	Stress	Form and dose	Method	Main physiological and biochemical responses	Yield and quality responses	Reference(s)
Tomato	Salinity (50-150 mM NaCl)	SeNPs, 10-20 mg mL^{-1}	Foliar	↑ chlorophyll a/b (%71-120); ↑ APX, CAT, SOD; ↓ MDA	↑ fruit weight %(13) and titratable acidity	Morales-Espinoza et al., 2019
Tomato	Salinity (150 mM NaCl)	Biogenic SeNPs, 5 mg L^{-1}	Foliar	↑ SOD, CAT, GPX; ↓ H_2O_2 and MDA; ↑ RWC, proline, sugars	↑ biomass, yield, and nutrient uptake	El-Badri et al., 2022
Tomato	Drought	SeNPs, 10-20 mg L^{-1}	Foliar	↑ chlorophyll, Fv/Fm, proline, sugars; ↓ MDA and H_2O_2,↑ SOD, CAT, POD	↑ height, biomass, yield, and WUE	Neysanian et al., 2024
Tomato	Drought	SeNPs, 75 ppm	Seed priming	↓ oxidative stress and lipid peroxidation; ↑ SOD, CAT, APX, proline, sugars	↑ germination, seedling growth, phenolics, flavonoids, and yield	Ishtiaq et al., 2023
Pepper	Salinity (25-50 mM NaCl)	SeNPs	Substrate / root zone	↑ phenolics, flavonoids, GSH, β-carotene; ↑ APX, GPX, CAT, PAL	↑ yield, fruit quality, and antioxidant capacity	Gonzalez-Garcia et al., 2021
Pepper	Salinity (100 mM NaCl)	Biogenic SeNPs, 5-20 mg L^{-1}	Foliar	↓ proline and MDA; ↑ chlorophyll, carotenoids, phenolics, APX, CAT, SOD	↑ growth, yield, and nutrient uptake	Kanber et al., 2025
Pepper	Salinity	SeNPs, 45 mg L^{-1}	Root zone	↑ peroxidase and pigments; ↓ MDA	↑ yield (%52.75), vitamin C, phenolics, and fruit quality	Sariñana-Navarrete et al., 2023
Pepper	Nutrient deficiency	SeNPs, 20-30 ppm / ionic Se, 20 ppm	Foliar	↑ N, P, K, Se, chlorophyll, carotenoids, antioxidant enzymes	↑ plant growth, yield, and fruit quality	Shedeed et al., 2023
Cucumber	Salinity (75 mM NaCl)	Na_2SeO_4, 0.5-1 mg L^{-1}	Foliar	↑ growth, chlorophyll, SOD, CAT, APX, proline; ↓ MDA and H_2O_2	↑ height, leaf area, yield, and quality	Shalaby et al., 2021

Crop	Stress	Form and dose	Method	Main physiological and biochemical responses	Yield and quality responses	Reference(s)
Cucumber	Salinity + heat	SeNPs, 25 mg L^{-1}	Foliar, 3 applications	↑ height, leaf area, chlorophyll, APX, CAT, SOD; ↓ MDA	↑ growth, yield, and stress tolerance	Shalaby et al., 2021
Lettuce	Drought	Na_2SeO_4, 5-10 µM	Foliar	↑ RWC, chlorophyll, phenolics, DPPH, FRAP, proline, sugars; ↓ MDA	↑ WUE, leaf yield, and quality	Sardar et al., 2022
Lettuce	Salinity	Selenate, 2-30 µM	Hydroponic	Growth promotion at 2-4 µM; phytotoxicity at 15-30 µM	Hormetic yield response	Hawrylak-Nowak, 2015
Common bean	Salinity	Na_2SeO_4,	Foliar	↑ nodulation, N fixation, and antioxidant enzymes	↑ growth, yield, and salinity tolerance	Hossain et al., 2022
Common bean	Nutrient deficiency	SeNPs, 20-30 ppm / ionic Se, 20 ppm	Foliar	↑ N, P, K, Se, pigments, and antioxidant enzymes	↑ growth, yield, and nutritional quality	Shedeed et al., 2023
Eggplant	Nutrient deficiency	SeNPs / ionic Se, 30-40 ppm	Foliar	↑ N, P, K, Se, pigments, and antioxidant enzymes	↑ seedling growth, yield, and fruit quality	Mahmoud et al., 2023
Broccoli	Nutrient deficiency	SeNPs / ionic Se, 30-40 ppm	Foliar	↑ nutrient status, glucosinolates, and phenolics	↑ head weight, yield, and nutritional quality	Abdalla et al., 2023
Onion	Nutrient deficiency	SeNPs / ionic Se, 30-40 ppm	Foliar	↑ N, P, K, Se, and antioxidant activity	↑ bulb weight, yield, and nutritional quality	El-Bassiony et al., 2023
Green bean	Nutrient deficiency	SeNPs, 20-30 ppm / ionic Se, 20 ppm	Foliar	↑ growth, N, P, K, Se, and nutrient uptake	↑ fresh-pod yield, pod quality, and nutrient content	Shedeed et al., 2023

Table 4 note: The effectiveness of Se and SeNPs depends on crop, stress type, formulation, dose, and application route. SeNPs have produced particularly strong improvements in tomato and pepper under salinity and have strengthened antioxidant protection in tomato and lettuce under drought. Biogenic SeNPs may provide comparable or superior responses at lower doses, while Se treatments in nutrient-deficient sandy soils have improved yield and mineral composition in eggplant, broccoli, onion, and green bean (El-Badri et al., 2022; El-Ramady et al., 2023; Shedeed et al., 2023).

6. SELENIUM AND NANO-SELENIUM APPLICATION METHODS AND DOSE-RESPONSE RELATIONSHIPS

The effectiveness of Se and SeNPs in enhancing plant stress tolerance varies substantially with application method, dose, timing, crop species, and environmental conditions (Fatima et al., 2024; Shriaiev et al., 2026). This section evaluates major application routes, the hormetic dose response, synergistic combinations, and practical optimization strategies.

6.1. Application Methods

Four principal application approaches are used in vegetable crops: foliar application, root-zone application through soil or hydroponic solution, seed priming, and postharvest treatment. Each method has distinct advantages, limitations, and modes of action (Table 5).

Table 5. Comparison of selenium and nano-selenium application methods

Method	Advantages	Limitations	Suitable crops	Indicative dose	Duration
Foliar application	• Rapid uptake • Targeted delivery • Less dependent on soil properties • Effective at low dose	• Lower efficacy in wind • Reapplication after rain may be needed • Leaf-scorch risk at high dose	Most vegetable crops	10-50 mg L^{-1} SeNPs 5-100 µM ionic Se	1-2 weeks
Root-zone application	• Prolonged response • May support root growth • Compatible with fertigation	• Soil adsorption and aggregation • More difficult dose adjustment • Slower response	Pepper, tomato, cucumber	20-50 ppm SeNPs 10-100 µM ionic Se	2-4 weeks
Seed priming	• Early-stage protection • Low dose • Simple treatment	• Primarily early-stage effects • Excess dose may inhibit germination	Tomato, lettuce, common bean	1-100 mg L^{-1} SeNPs 10-100 µM ionic Se	Early development
Hydroponic application	• Controlled environment • Precise dosing • Uniform distribution	• Limited to soilless systems • Higher infrastructure cost	Tomato, lettuce, cucumber	1-20 mg L^{-1} SeNPs 1-50 µM ionic Se	Continuous
Postharvest treatment	• Extends shelf life • Maintains quality • May suppress pathogens	• Direct contact with produce • Residue and consumer-safety concerns	Tomato, pepper, strawberry	50-150 mg L^{-1} SeNPs	1-4 weeks

Source: Compiled from El-Ramady et al. (2020), Avellan et al. (2021), Fatima et al. (2024), and Shriaiev et al. (2026).

Table 5 note: Foliar application is generally the most rapid and widely used method. Seed priming is particularly useful for early-stage protection, whereas postharvest treatments are intended primarily to maintain harvested-product quality.

6.1.1. Foliar Application

Foliar spraying is among the most common and effective approaches for applying Se and SeNPs (Fatima et al., 2024; Shriaiev et al., 2026). Solutions are delivered directly to the leaf surface and absorbed through the cuticle, stomata, and hydathodes.

Advantages:

- **Rapid uptake:** Se and SeNPs can be absorbed rapidly through leaf-surface structures (Avellan et al., 2021).
- **Targeted delivery:** the treatment can be directed toward metabolically active foliage and, indirectly, toward developing fruits.
- **Reduced dependence on soil properties:** foliar uptake is less directly constrained by soil pH, salinity, and organic matter (El-Ramady et al., 2020).
- **Efficacy at relatively low doses:** foliar application may be effective at lower total rates than soil application (Shriaiev et al., 2026).

Limitations:

- Efficacy declines under windy conditions;

- Rain may wash the treatment from leaves and necessitate reapplication; and
- Excessive concentrations may cause leaf scorch.

Suggested protocol:

1. Prepare the Se or SeNP suspension with clean, low-salinity water.
2. Spray both adaxial and abaxial leaf surfaces to uniform wetness without excessive runoff.
3. Apply in the early morning (approximately 06:00-09:00) or late afternoon (approximately 17:00-19:00).
4. Avoid intense solar radiation, strong wind, and imminent rainfall.
5. Adjust the 7-15-day interval according to crop-specific recommendations and plant response.

6.1.2. Root-Zone Application through Soil or Hydroponic Solution

In root-zone application, Se or SeNPs are introduced through irrigation water, a nutrient solution, or a soil drench (El-Ramady et al., 2020). Selenium absorbed by roots is transported to shoots predominantly through the xylem (Fatima et al., 2024).

Advantages:

- **Prolonged supply:** Se can remain available in the rhizosphere or nutrient solution and support a longer response (El-Ramady et al., 2023).
- **Root development:** suitable Se doses may promote root growth and branching (El-Badri et al., 2022).
- **Potential contribution to stress management:** Se may support ion balance in saline or metal-contaminated growth media (Hussain et al., 2020).

Limitations:

- adsorption and aggregation of SeNPs on soil particles may restrict uptake (Fatima et al., 2024); and
- effective dosing depends strongly on soil texture, pH, organic matter, salinity, and irrigation management.

Suggested protocol:

1. Use biologically coated or chitosan-modified formulations when appropriate to improve SeNP stability (Sheikhalipour et al., 2021).
2. Disperse the treatment uniformly in irrigation water or nutrient solution.
3. Apply close to the active root zone.
4. In hydroponic systems, monitor Se concentration and solution quality regularly.

6.1.3. Seed Priming

Seed priming involves soaking seeds in a Se or SeNP solution for a defined period before sowing (El-Badri et al., 2022; Fatima et al., 2024). It is intended to support germination and early seedling establishment.

Advantages:

- **Rapid and uniform germination:** Se may activate enzymes associated with reserve mobilization, including α-amylase (El-Badri et al., 2022).
- **Early protection:** primed seedlings may show improved tolerance during establishment (Ishtiaq et al., 2023).
- **Low-dose efficacy:** effective concentrations may be as low as 1-10 mg L^{-1} for some crops and formulations (Fatima et al., 2024).

Limitations:

- the protective effect may be concentrated in early development; and
- excessive dose or soaking duration can inhibit germination.

Suggested protocol:

1. Prepare a crop-appropriate Se or SeNP solution, generally within 1-100 mg L^{-1} for SeNPs.
2. Soak seeds for approximately 4-8 h, depending on species and seed size.
3. Shade-dry the seeds to a sowable condition.
4. Monitor germination percentage, emergence uniformity, and early seedling growth.

6.1.4. Postharvest Applications

Se and SeNPs may also be used after harvest to extend shelf life, preserve quality, and increase resistance to postharvest pathogens (Tran et al., 2023; Shriaiev et al., 2026).

Potential advantages:

- Extension of storage life (Liu et al., 2022);
- Maintenance of color, texture, and nutritional quality; and
- Suppression of postharvest pathogens (Salem et al., 2022).

Limitations:

- Direct contact with edible produce; and
- The need for rigorous dose, residue, and consumer-safety assessments.

Suggested protocol:

1. Prepare an SeNP treatment, often within 50-150 mg L^{-1} in experimental studies.
2. Apply by dipping or spraying.
3. Where appropriate, incorporate SeNPs into an edible film or hydrogel coating (Tran et al., 2023).
4. Store produce under the recommended cold-chain conditions.

6.2. Dose-Response Relationships: Hormesis and Optimization

The biological response to Se is biphasic. Low doses may reinforce antioxidant defense, protect photosynthesis, and improve osmotic adjustment, whereas excessive doses increase ROS production and phytotoxicity (Hasanuzzaman et al., 2020; Fatima et al., 2024; Tsivileva, 2025).

6.2.1. Low Dose: Stimulatory and Protective Effects

Low Se concentrations, often within 1-10 mg L^{-1} or the low micromolar range depending on formulation, may produce the following responses:

- Increased SOD, CAT, APX, GPX, and GR activities;
- Greater accumulation of proline, soluble sugars, phenolics, and flavonoids;
- Maintenance of chlorophyll and carotenoids;
- Improved ion homeostasis through lower Na^+ and higher K^+ uptake; and

• Increased growth and yield.

6.2.2. Optimum Dose

The optimum dose depends on crop species, formulation, application method, stress intensity, growth stage, and environmental conditions (Table 6). It is the dose that provides maximum benefit without visible toxicity or unacceptable Se accumulation.

Table 6. Indicative optimum selenium and nano-selenium doses reported for selected vegetable crops

Crop	Stress	Se form	Indicative optimum dose	Method	Reference
Tomato	Salinity / drought	SeNPs	10-20 mg L^{-1}	Foliar	Hernández-Hernández et al., 2019; Neysanian et al., 2020
Tomato	Drought	SeNPs	75 ppm	Seed priming	Ishtiaq et al., 2023
Pepper	Salinity	SeNPs	20-30 mg L^{-1}	Foliar	Kanber et al., 2025
Pepper	Salinity	Na_2SeO_3	20 ppm	Foliar	Shedeed et al., 2023
Pepper	Salinity	SeNPs	20-30 ppm	Root zone	Shedeed et al., 2023
Cucumber	Salinity / heat	SeNPs	25 mg L^{-1}	Foliar	Shalaby et al., 2021
Cucumber	Salinity	Na_2SeO_4	0.5-1 mg L^{-1}	Foliar	Shalaby et al., 2021
Lettuce	Drought	Na_2SeO_4	5-10 µM	Foliar	Sardar et al., 2022
Lettuce	Salinity	Selenate	2-4 µM	Hydroponic	Hawrylak-Nowak, 2015
Common bean	Nutrient deficiency	SeNPs	20-30 ppm	Foliar	Shedeed et al., 2023
Common bean	Nutrient deficiency	Na_2SeO_3	20 ppm	Foliar	Shedeed et al., 2023
Eggplant	Nutrient deficiency	SeNPs / ionic Se	30-40 ppm	Foliar	Mahmoud et al., 2023
Broccoli	Nutrient deficiency	SeNPs / ionic Se	30-40 ppm	Foliar	Abdalla et al., 2023
Onion	Nutrient deficiency	SeNPs / ionic Se	30-40 ppm	Foliar	El-Bassiony et al., 2023
Green bean	Nutrient deficiency	SeNPs	20-30 ppm	Foliar	Shedeed et al., 2023

Source: Compiled from the studies summarized in Table 4.

Table 6 note: The values shown are indicative experimental optima rather than universal recommendations. SeNPs are often effective at lower concentrations than bulk or ionic Se, but the safe and effective rate must be verified for each crop, formulation, and production environment.

6.2.3. High Dose: Toxic and Pro-Oxidant Effects

Excessive Se concentrations, frequently above approximately 50 mg L^{-1} or 100 μM depending on crop and formulation, may cause:

• Greater ROS production and oxidative stress (Tsivileva, 2025);
• Increased MDA and lipid peroxidation (Haghighi et al., 2014);
• Chlorophyll loss and photosynthetic inhibition (Morales-Espinoza et al., 2019);
• Growth inhibition and yield loss (Neysanian et al., 2020); and
• Phytotoxic symptoms including chlorosis, necrosis, leaf scorch, and root injury.

6.2.4. Practical Dose-Optimization Guidance

1. **Start low:** begin with a conservative concentration, often 5-0 mg L^{-1} for experimental foliar SeNP treatments.
2. **Observe carefully:** evaluate leaf color, growth, water status, and stress symptoms after two or three treatments.
3. **Increase gradually:** increase the rate only when the response is positive and no toxicity is evident.
4. **Use crop-specific evidence:** consult dose ranges reported for the crop, growth stage, stress, and application route.
5. **Respond to toxicity:** reduce the dose or discontinue application if chlorosis, necrosis, leaf scorch, root injury, or growth suppression occurs.

6.3. Synergistic Effects and Combination Strategies

Combining Se or SeNPs with other elements or biopolymers may produce complementary or synergistic stress-mitigation effects (Fatima et al., 2024; Shriaiev et al., 2026).

6.3.1. Selenium and Silicon Synergy

Combined Se and Si application can enhance tolerance to drought and salinity (Zahedi et al., 2020; Shriaiev et al., 2026). Se/SiO_2 nanostructures protected photosynthetic pigments, enhanced antioxidant enzymes, and improved fruit quality in drought-stressed strawberry (Zahedi et al., 2020). Potential complementary effects include:

• More effective ROS scavenging;
• Improved osmotic adjustment; and

• Stronger ion homeostasis (Hussain et al., 2020).

6.3.2. Selenium and Chitosan Synergy

Chitosan is a natural biopolymer that can be combined with SeNPs to improve formulation stability and plant response (Sheikhalipour et al., 2021, 2023). Chitosan-selenium nanoparticles improved growth, photosynthesis, and antioxidant defense in salt-stressed bitter melon (Sheikhalipour et al., 2021). Potential benefits include:

• Facilitated SeNP uptake and transport;
• Increased antioxidant enzyme activity; and
• Enhanced proline accumulation (Azimi et al., 2021).

6.3.3. Selenium in Combination with Other Elements

Se may interact synergistically with micronutrients such as Zn, Cu, and Fe (Shriaiev et al., 2026). Combined Se and Cu nanoparticles enhanced tomato resistance to early blight caused by *Alternaria solani* (Quiterio-Gutiérrez et al., 2019). Co-application of Se and Zn may also improve nutrient uptake and stress tolerance, although dose compatibility and residue safety require evaluation (Fatima et al., 2024).

6.4. The 4R Principle for Successful Application

The 4R nutrient-management concept provides a useful framework for practical Se and SeNP use (El-Ramady et al., 2023).

1. Right source: Select nano-selenium when lower acute toxicity and high bioavailability are priorities; consider biogenic SeNPs for environmentally compatible formulations; use selenate or selenite only with strict dose control.

2. Right rate: Use crop- and stress-specific evidence, begin conservatively, and monitor for toxicity.

3. Right time: Apply in the early morning or late afternoon, preferably before stress or during the early stress phase, and avoid application immediately before rainfall.

4. Right place: Ensure uniform coverage of both leaf surfaces during foliar treatment, target the active root zone during soil application, and mix uniformly in hydroponic nutrient solution.

6.5. Pre-Application Checklist

- Which stress is being managed: drought, salinity, heat, nutrient deficiency, or a combination?
- Which vegetable crop, cultivar, growth stage, and production system are involved?
- Which application route will be used: foliar, root zone, seed priming, hydroponic, or postharvest?
- Has the appropriate Se form been selected: ionic Se, SeNPs, or biogenic SeNPs?
- Has a crop-and formulation-specific dose been identified?
- Is the timing suitable with respect to phenology, solar radiation, wind, and rainfall?
- Are personal-protective measures in place, including gloves, a mask, and eye protection?
- Is there a plan to monitor plant response, phytotoxicity, Se accumulation, and product residues?

6.6. Summary of Application Strategies

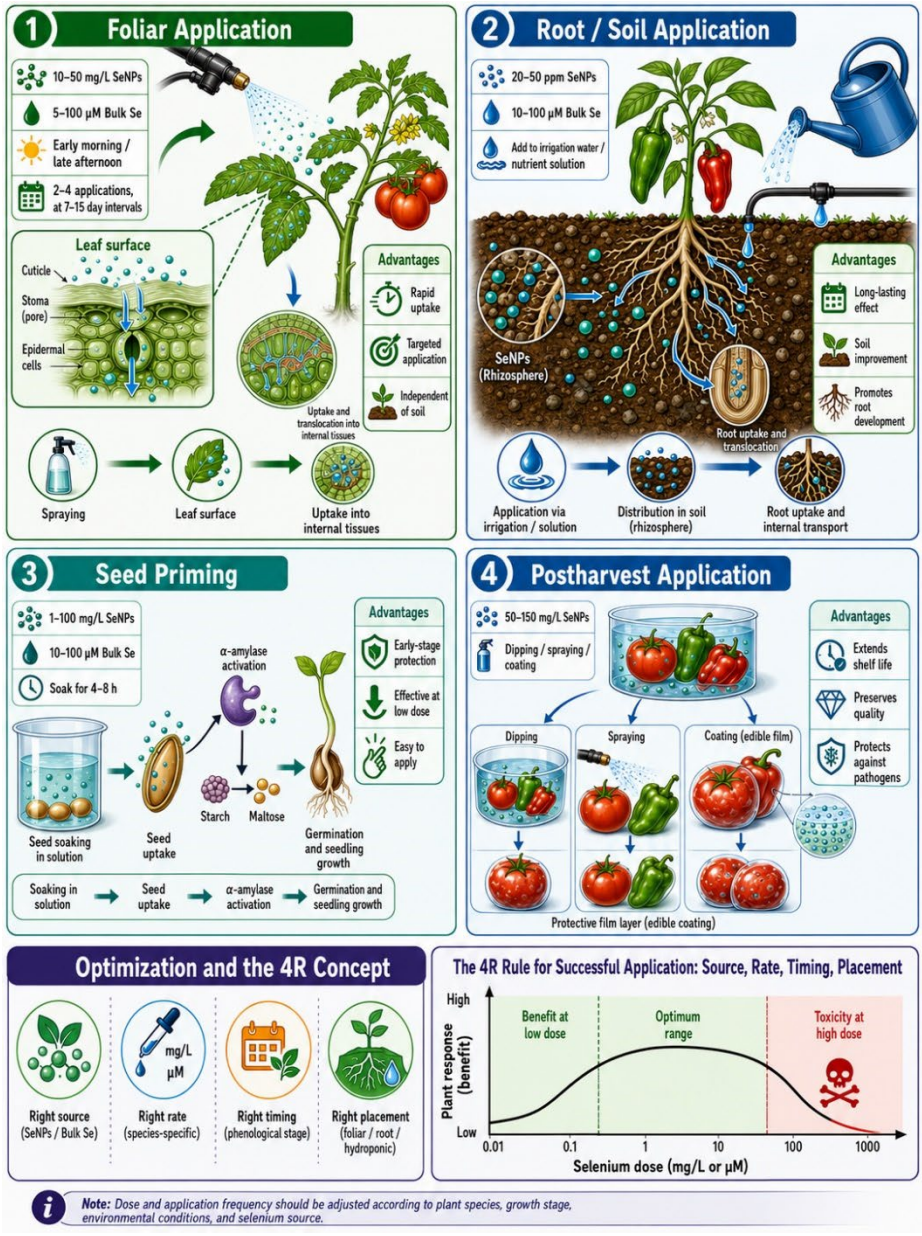

Figure 4. Major selenium and nano-selenium application routes, the 4R framework, and the hormetic dose-response relationship.

7. CONCLUSION

This review demonstrates that Se and SeNP applications can enhance the tolerance of vegetable crops to drought and salinity. The evidence indicates that selenium, particularly when supplied as an optimized nano-formulation, has substantial potential as a component of sustainable stress-management strategies in vegetable production.

7.1. Key Findings

1. At low and optimized doses, Se and SeNPs mitigate drought and salinity injury by strengthening antioxidant defense, protecting chlorophyll and PSII function, promoting osmotic adjustment, and maintaining Na^+, K^+, Ca^{2+}, and Mg^{2+} homeostasis.
2. Compared with ionic selenate and selenite, SeNPs may provide higher bioavailability, lower acute toxicity, controlled release, and stronger antioxidant responses. Biogenic SeNPs are especially promising because their synthesis can be environmentally compatible and their coatings may improve stability and uptake.
3. Studies in tomato, pepper, cucumber, lettuce, common bean, eggplant, broccoli, onion, and green bean have reported improvements in physiological performance, yield, and quality. Yield increases of up to approximately %21 in tomato and %52.75 in pepper have been reported under specific experimental conditions.
4. The optimum dose varies with crop, formulation, stress intensity, and application method, but many experimental foliar SeNP treatments fall within 10-50 mg L^{-1}. Because Se follows a hormetic response, the effective dose must be optimized carefully.
5. Foliar treatment is generally the most rapid and widely used method, while seed priming, root-zone delivery, hydroponic application, and postharvest treatments are useful for specific objectives.
6. Combinations of Se with silicon, chitosan, or selected micronutrients may further enhance stress tolerance, but their agronomic compatibility and safety must be confirmed.

7.2. Practical Implications

- **For growers:** Se and SeNP treatments may help reduce yield and quality losses in drought-prone or salt-affected production systems.

Foliar application is generally the most practical route, whereas seed priming may improve early seedling resilience. Commercial use should follow crop-specific labels, local field validation, residue limits, and occupational-safety requirements.

- **For researchers:** Future studies should address long-term effects, interactions with soil microbial communities, molecular mechanisms, particle fate, field-scale validation, food-chain transfer, residue behavior, and biofortification efficiency across cultivars and production environments.
- **For policymakers and extension services:** Guidance for Se-based agricultural products should integrate efficacy, environmental fate, worker protection, consumer safety, and traceability. Extension programs are needed to translate validated findings into safe and practical recommendations.

In conclusion, selenium and especially nano-selenium may contribute to sustainable vegetable production under drought and salinity when used within a scientifically validated dose range and as part of an integrated crop-management strategy. Appropriate formulation, timing, placement, monitoring, and knowledge transfer are essential for obtaining agronomic benefits while avoiding phytotoxicity and excessive Se accumulation.

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CHAPTER 18

ADVANCED POSTHARVEST MANAGEMENT OF STONE FRUITS: THE ROLE OF POLYAMINES, SALICYLIC ACID, AND CONTROLLED AND MODIFIED ATMOSPHERE TECHNOLOGIES IN QUALITY PRESERVATION

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1. INTRODUCTION

Stone fruits such as cherries, plums, peaches, nectarines, and apricots are horticultural commodities of considerable economic importance for fresh consumption. However, their rapid postharvest deterioration creates major challenges during long-distance transportation, storage, and marketing. In sweet cherries in particular, softening, water loss, color changes, and decay are among the main quality problems limiting storage and shelf life (Ağlar et al., 2017; Kurubaş et al., 2018).

Fruit metabolism does not cease completely after harvest; respiration, ripening, senescence, and cell-wall metabolism continue. These processes directly affect firmness, color, acidity, weight, and consumer acceptance. The primary objective of postharvest management is therefore not to eliminate physiological activity entirely, but to slow quality deterioration as much as possible.

Cold storage is one of the core technologies used for the postharvest preservation of stone fruits. Nevertheless, cold storage alone cannot prevent every quality loss that may arise during prolonged storage and transportation. Consequently, increasing attention has been directed toward complementary approaches such as polyamines, salicylic acid, and modified-atmosphere systems.

Among polyamines, putrescine, spermidine, and spermine are of particular interest because of their relationships with fruit senescence, ethylene metabolism, and cellular integrity. A direct study on 'Angelino' plum showed that putrescine treatment reduced ethylene production and delayed fruit softening (Khan et al., 2007).

Similarly, postharvest salicylic acid treatment has produced favorable results in sweet cherry by delaying ripening and helping to preserve bioactive compounds (Valero et al., 2011).

Modified atmosphere packaging is also an important postharvest technology for cherries, particularly with respect to limiting weight loss and decay and preserving color, firmness, and certain bioactive constituents (Ağlar et al., 2017; Kurubaş et al., 2018).

The objective of this review is to explain the principal mechanisms of postharvest quality loss in stone fruits and to evaluate the potential of

polyamines, salicylic acid, and controlled/modified atmosphere applications for quality preservation in light of directly relevant experimental evidence.

2. POSTHARVEST PHYSIOLOGY AND DETERIORATION MECHANISMS IN STONE FRUITS

Although harvest separates the fruit from the parent plant, it does not terminate physiological activity. Respiration and various metabolic processes continue in fruit tissues after harvest. Their rates are influenced by factors such as temperature, fruit maturity, and storage atmosphere.

Fruit softening is one of the major forms of quality deterioration in stone fruits. Changes in cell-wall and middle-lamella components reduce fruit firmness. In particular, changes in the activity of enzymes involved in pectic-substance degradation are associated with a loss of structural integrity in fruit tissues.

In 'Angelino' plum, putrescine treatment reduced ethylene production and restricted the activities of exo-polygalacturonase, endo-polygalacturonase, pectin esterase, and endo-1,4-beta-glucanase, which are associated with fruit softening. These findings indicate that polyamines may help preserve firmness through effects on both ethylene and cell-wall metabolism (Khan et al., 2007).

Ethylene is an important regulator of ripening and senescence in many fruits, but its role is neither identical nor equally pronounced in all stone-fruit species. Species- and cultivar-specific differences must therefore be considered.

Oxidative processes and changes in membrane integrity may also contribute to postharvest quality deterioration. These changes can lead to discoloration, tissue breakdown, and losses of certain bioactive compounds. Modern postharvest technologies therefore target multiple quality parameters rather than softening alone.

3. ROLE OF POLYAMINES IN POSTHARVEST QUALITY PRESERVATION

Polyamines, particularly putrescine, spermidine, and spermine, are naturally occurring low-molecular-weight polycationic compounds in plant cells. In addition to growth and development, they are involved in stress responses and senescence.

One important aspect of polyamines in postharvest quality preservation is their relationship with ethylene metabolism. Because the biosynthetic pathways of polyamines and ethylene share metabolic connections, reciprocal interactions may occur between the two systems.

One of the clearest experimental examples of this relationship in stone fruits has been reported for 'Angelino' plum. Khan et al. (2007) treated plum fruit with different concentrations of putrescine, stored them at 0 +/- 1 °C, and found that putrescine reduced ethylene production during storage. Treatment-related changes were also observed in ACS and ACO activities and ACC content.

Another important result of that study was the reduction in enzyme activities associated with fruit softening. Decreases in exo- and endo-polygalacturonase, pectin esterase, and endo-1,4-beta-glucanase activities contributed to prolonged maintenance of fruit firmness (Khan et al., 2007).

These results support the interpretation of putrescine not only as a treatment that can suppress ethylene production, but also as a postharvest regulator capable of influencing cell-wall metabolism.

The effects of polyamines should not be expected to be identical across all fruit species. Treatment dose and timing, fruit maturity, cultivar, and storage temperature can alter the outcome. Accordingly, optimum conditions identified for one species should not be transferred directly to another species or cultivar.

Future research on spermidine should include direct comparisons among different stone fruits, including cherry, plum, peach, and apricot. Such studies would provide more reliable evidence of species- and cultivar-specific effectiveness.

4. ROLE OF SALICYLIC ACID IN POSTHARVEST QUALITY MANAGEMENT

Salicylic acid is an important signaling molecule associated with plant defense and stress responses. Postharvest research has examined its potential effects on fruit ripening, antioxidant properties, and selected quality parameters.

Valero et al. (2011) directly evaluated postharvest salicylic acid treatment in 'Cristalina' and 'Prime Giant' sweet cherries. Fruit harvested at commercial maturity were treated with 1 mM salicylic acid and stored under

cold conditions for 20 days. The treatment delayed postharvest ripening and contributed to longer preservation of quality attributes.

The same study reported more favorable changes in total phenolics, anthocyanins, and total antioxidant activity throughout storage in salicylic-acid-treated fruit. These findings suggest that salicylic acid may help preserve not only the physical quality of sweet cherries but also their bioactive constituents (Valero et al., 2011).

Salicylic acid has also been investigated in sweet cherry as a pre- and postharvest treatment for induced resistance to storage diseases. Yao and Tian (2005) reported differences associated with application timing. Nevertheless, evidence from a single species and a limited set of treatment conditions is insufficient to regard salicylic acid as a standard disease-control method for all stone fruits.

Species, cultivar, concentration, application timing, and storage conditions must therefore be considered together when salicylic acid treatments are evaluated.

5. CONTROLLED AND MODIFIED ATMOSPHERE APPLICATIONS

Controlled atmosphere (CA) storage and modified atmosphere packaging (MAP) are two technologies based on altering oxygen and carbon dioxide concentrations around the product, although they differ in the way these concentrations are controlled. In CA systems, gas composition is monitored and actively adjusted throughout storage; in MAP, the atmosphere develops through the balance between product respiration and package gas permeability. Both approaches aim to slow respiration and senescence without inducing anaerobic metabolism.

Sweet cherry is one of the stone fruits most extensively studied in relation to MAP. Research on '0900 Ziraat' cherry reported that MAP substantially reduced weight loss during cold storage and shelf life and produced lower decay rates than the control treatment. Some quality attributes, including color and vitamin C content, were also better preserved (Ağlar et al., 2017).

Similarly, Kurubaş et al. (2018) compared different MAP materials for '0900 Ziraat' cherries and reported that Xtend® bags limited weight loss and helped preserve firmness, titratable acidity, color, and sensory quality at 0 °C

and 90-95% relative humidity. Under the specific conditions of that study, fruit could be stored for up to 50 days; this duration should not be generalized directly to other cultivars or packaging systems.

These findings indicate that appropriately designed MAP systems can reduce water loss and help preserve selected physicochemical quality attributes in cherries. Their effectiveness depends on cultivar, maturity, precooling, package permeability, product load, and temperature management.

The gas composition created by MAP must be compatible with the respiratory characteristics of the product. Because excessively low oxygen or excessively high carbon dioxide can cause undesirable metabolic changes, package gas permeability, product respiration rate, and storage temperature must be evaluated together.

The combined effects of oxygen, carbon dioxide, and temperature on sweet-cherry metabolism demonstrate that atmospheric composition should be adjusted to product physiology (Petracek et al., 2002). It is therefore scientifically inappropriate to recommend a single ideal MAP gas composition or packaging material for all stone fruits.

Table 1. Principal target effects and evidence limitations of the applications reviewed

Application	Product directly evaluated	Principal effects observed	Primary limitation
Putrescine	'Angelino' plum	Reduced ethylene production, cell-wall enzyme activity, and softening	Responses depend on dose, maturity, and cultivar.
Salicylic acid	Sweet cherry	Delayed ripening; preservation of phenolics, anthocyanins, and antioxidant capacity	Cannot be generalized to all stone-fruit species.
MAP	'0900 Ziraat' and 'Sams' cherries	Management of weight loss, firmness, color, and selected deterioration indicators	Gas balance is specific to package, product, and temperature.
Combined systems	Limited direct evidence	Potentially complementary mechanisms	Synergy must be confirmed through factorial experiments.

6. COMBINED POSTHARVEST TECHNOLOGIES

Because postharvest quality deterioration results from the combined action of numerous physiological and biochemical processes, combining

technologies that act through different mechanisms represents an important research area.

Polyamines are primarily associated with ethylene metabolism and softening; salicylic acid with ripening, defense responses, and preservation of bioactive compounds; and MAP with gas exchange, water loss, and the storage atmosphere. These distinct modes of action suggest that the treatments could theoretically complement one another.

Current literature does not, however, establish that a polyamine + salicylic acid + MAP combination is the most effective method for all stone fruits. Such a conclusion would require experimental studies directly comparing combined treatments.

Future studies should evaluate the individual and combined effects of polyamines, salicylic acid, MAP, and cold storage using factorial experimental designs. This approach could identify potential synergistic or antagonistic interactions among treatments.

Particularly under near-commercial conditions, simultaneous evaluation of fruit firmness, weight loss, decay incidence, color, total soluble solids, titratable acidity, phenolic compounds, antioxidant activity, and consumer acceptance would help establish the practical commercial value of these treatments.

7. COMMERCIAL APPLICABILITY AND FUTURE PERSPECTIVES

Preserving fruit quality is not the only prerequisite for the commercial use of a postharvest technology. The treatment must also be economical, readily integrated into the existing cold chain, amenable to standardized application, and reliable under commercial storage conditions.

For MAP, package gas permeability, fruit respiratory characteristics, storage temperature, and relative humidity should be evaluated together. Studies on '0900 Ziraat' cherry indicate that appropriate MAP treatments can reduce quality losses during storage and marketing (Ağlar et al., 2017; Kurubaş et al., 2018).

Polyamine and salicylic acid treatments require standardization of concentration, application timing, and application method. In particular,

findings obtained under laboratory conditions need to be validated at commercial scale.

Important future research areas include comparing different species and cultivars under the same experimental conditions, identifying optimum treatment doses, and monitoring quality changes during storage using multiple parameters.

Economic analyses, evaluation of treatment cost in relation to the resulting quality benefit, and determination of consumer acceptance are also important for the wider commercial adoption of these technologies.

8. CONCLUSIONS AND RECOMMENDATIONS

Postharvest quality losses in stone fruits result from interrelated processes including respiration, ethylene metabolism, cell-wall modification, changes in membrane integrity, water loss, and microbial growth. A comprehensive quality-management strategy should therefore consider complementary technologies that act through different mechanisms rather than relying on temperature control alone.

Among polyamines, putrescine is particularly noteworthy for its potential to delay fruit softening and restrict ethylene metabolism in stone fruits. Direct experimental evidence from 'Angelino' plum showed that putrescine treatment could reduce ethylene production and the activities of several enzymes associated with fruit softening (Khan et al., 2007).

Salicylic acid is a promising treatment for delaying postharvest ripening and preserving bioactive compounds and antioxidant capacity, particularly in sweet cherry. Valero et al. (2011) directly demonstrated that salicylic acid treatment could favorably influence quality changes in sweet cherries during storage.

MAP also has considerable potential to reduce weight loss and decay and to preserve firmness and selected chemical quality attributes, especially in sweet cherry. Studies on '0900 Ziraat' cherry indicate that suitable MAP systems can support prolonged cold storage and subsequent shelf life (Ağlar et al., 2017; Kurubaş et al., 2018).

Nevertheless, the available literature does not show that polyamine, salicylic acid, and MAP treatments are equally effective in every stone fruit. Future research should therefore be species- and cultivar-specific, optimize

treatment doses and timing, and directly compare combinations of different technologies.

In conclusion, future postharvest management of stone fruits should be based not on the presumed superiority of a single treatment, but on product- and cultivar-specific integration of the cold chain, appropriate biological regulators, and optimized atmosphere management. Establishing commercial applicability will require joint consideration of technological effectiveness, economic cost, ease of application, consumer acceptance, and performance under commercial storage conditions.

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CHAPTER 19

PRE-BREEDING APPROACHES IN OLIVE AND THE USE OF GENETIC RESOURCES FOR BREEDING

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INTRODUCTION

Olive (*Olea europaea* L.) is among the most extensively cultivated perennial fruit species in the Mediterranean basin. Its wild, feral, and cultivated forms are widely distributed throughout this region (Fanelli et al., 2022; Debbabi et al., 2022). The genus *Olea* is divided into three subgenera: *Tetrapilus, Paniculatae*, and *Olea*. The subgenus *Olea* comprises both cultivated and wild olive forms. Cultivated olives are commonly classified as *Olea europaea* L. subsp. *europaea*, whereas wild olives are referred to as *Olea europaea* L. subsp. *oleaster* or subsp. *sylvestris*. Both cultivated and wild olives are diploid species with 2n = 46 chromosomes. Several studies have identified *Olea europaea* subsp. *europaea* var. *sylvestris* as a wild relative of cultivated olive genotypes (Rugini et al., 2011; Chiappetta & Muzzalupo, 2012; Bellini et al., 2008; Besnard & Rubio de Casas, 2016; Fanelli et al., 2022; Falek et al., 2022; Acar, 2024a). Wild olive trees are still found in some Mediterranean forest regions, where they represent important natural genetic resources (Lumaret & Ouazzani, 2001; Bombarely et al., 2021).

In addition to its value as a genetic resource, olive cultivation has long been an integral part of Mediterranean landscapes and cultural heritage. Olive-growing systems contribute not only to agricultural production but also to biodiversity and other ecological functions. As olive cultivation continues to intensify, sustainable management has become increasingly important for maintaining these ecological values while supporting agricultural productivity (Raz et al., 2024).

According to the International Olive Council (IOC), olive cultivation is mainly concentrated in the Mediterranean Basin, where about 95% of global olive oil production takes place. Spain, Türkiye, Italy, Greece, and Tunisia are among the leading producing countries. Global olive oil production reached approximately 3.57 million tons in the 2024/25 crop year and is expected to reach about 3.44 million tons in 2025/26 (IOC, 2026). Olive cultivation covers more than 11 million hectares worldwide, with most olive-growing areas still located in the Mediterranean region (IOC, 2026). Although olive originated in this region, its cultivation has expanded to other parts of the world, including South Africa, Australia, China, Japan, and Latin America (Figure 1). This expansion is associated with the species' adaptability to different environmental

conditions and the increasing global demand for olive oil due to its nutritional and health-promoting properties (Hatzopoulos et al., 2002; IOC, 2026).

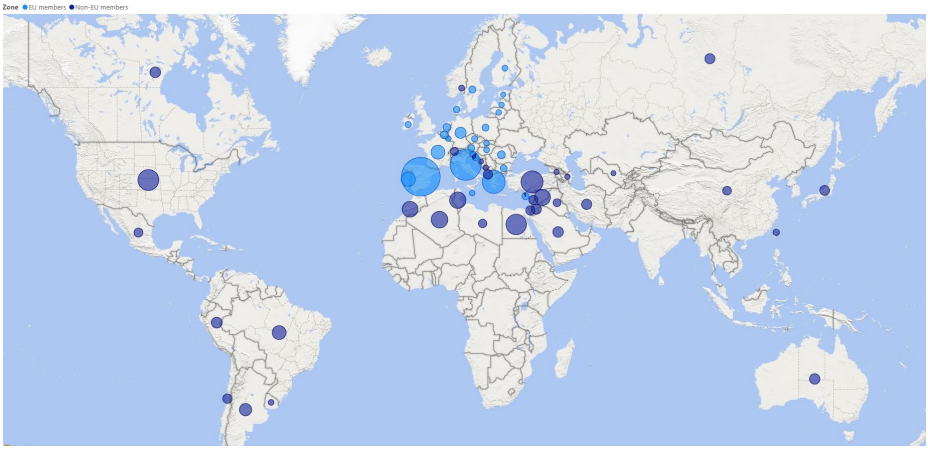

Figure 1. Worldwide Distribution of Olive Cultivation. Reproduced from IOC (2026).

Olive production in 2024 was concentrated in a relatively small number of countries (Figure 2). Spain remained the largest producer, followed by Türkiye and Italy, whereas Portugal, Egypt, Morocco, Tunisia, Algeria, and the Syrian Arab Republic also contributed substantially to total production. These figures further demonstrate the dominant position of Mediterranean countries in global olive cultivation and production (FAOSTAT, 2026).

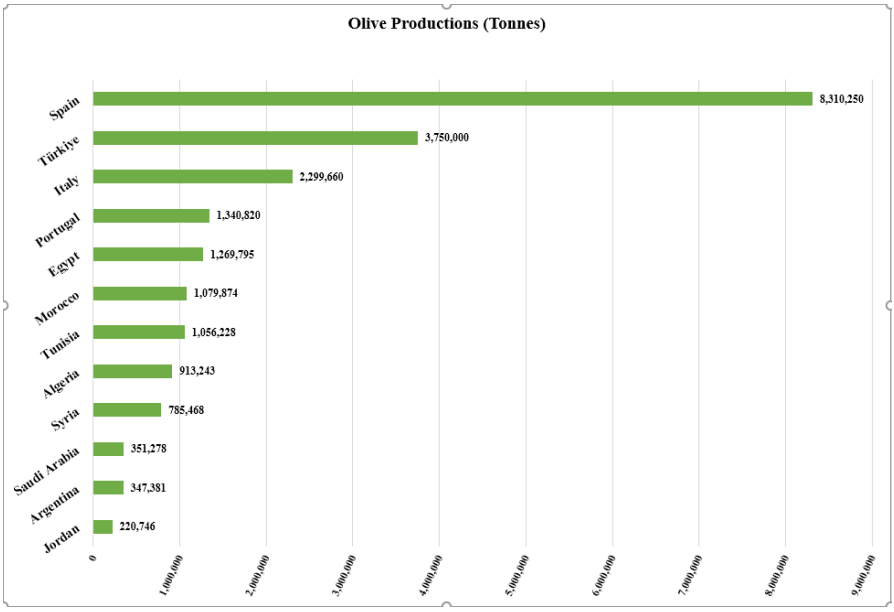

Figure 2. Olive Production in Major Olive-Producing Countries in 2024 (FAOSTAT, 2026).

Genetic diversity plays a fundamental role in crop adaptation to changing environmental conditions by enhancing tolerance to both biotic and abiotic stresses, while also supporting the maintenance of important agronomic traits, including quality characteristics (Salgotra & Chauhan, 2023). The effective utilization of this genetic diversity is therefore essential for achieving sustained genetic improvement in plant breeding.

Although olive exhibits substantial genetic diversity, its utilization in breeding programs remains limited due to several biological constraints, including a prolonged juvenile phase (de la Rosa et al., 2016) and high levels of heterozygosity (Rugini et al., 2016a). These intrinsic characteristics lengthen breeding cycles and reduce the efficiency of selection in conventional breeding systems.

Furthermore, most modern olive cultivars are thought to have originated from a relatively narrow genetic base (Besnard et al., 2018), which may limit the genetic variation available for future breeding. These limitations suggest that conventional breeding alone is unlikely to fully exploit the existing genetic diversity in olive.

For this reason, pre-breeding has gained increasing importance as a bridge between genetic resources and applied breeding. It supports the

identification, characterization, and transfer of valuable alleles from wild relatives, feral forms, and underutilized germplasm into breeding materials. This review focuses on olive genetic resources and their use in breeding programs, with particular emphasis on pre-breeding approaches, and discusses current challenges and future perspectives for their effective utilization in olive breeding.

OLIVE GENETIC RESOURCES

Olive (*Olea europaea* L.) is among the earliest domesticated tree crops and originated from the natural forests of the Mediterranean Basin (Besnard et al., 2001; Lavee, 2013). Domestication probably occurred in the Middle East and Central Mediterranean approximately 5,500 years BP, followed by the gradual spread of cultivation across the Mediterranean region (Rallo et al., 2018) (Figure 3). Despite its long cultivation history, several aspects of olive domestication and the evolutionary relationships between cultivated and wild olives remain unclear (Diez et al., 2016; Besnard et al., 2018). This uncertainty is partly due to the difficulty of distinguishing genuinely wild olives from feral forms, which complicates studies of olive domestication and the assessment of genetic resources (Besnard et al., 2018). Therefore, the characterization and conservation of olive genetic resources are essential for their effective use in breeding and for the development of cultivars with improved productivity, oil quality, and tolerance to biotic and abiotic stresses (Debbabi et al., 2022).

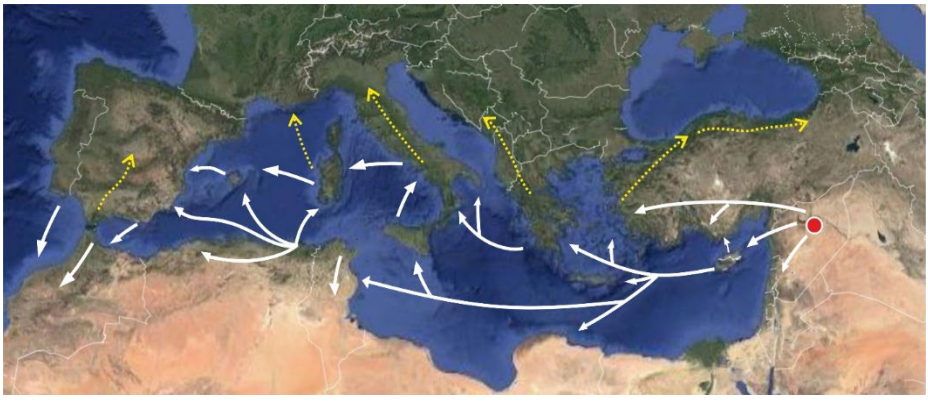

Figure 3. Schematic representation of the spread of olive domestication across the Mediterranean Basin, adapted from Lavee (2013).

The importance of olive genetic resources is reflected in the remarkable diversity of olive germplasm. More than 2,000 olive cultivars have been reported worldwide (Fanelli et al., 2022; Galatali et al., 2021; Acar, 2024b). However, determining the exact number of olive cultivars remains challenging because of the occurrence of synonymy, homonymy, and the continued discovery of previously undocumented germplasm across Mediterranean countries (Klepo et al., 2024). Despite this extensive diversity, only 139 cultivars account for approximately 85% of global olive oil production (IOC, 2026), indicating that a large proportion of olive genetic resources remains underutilized in modern production systems. To safeguard these resources, olive cultivars are conserved in more than 100 collections across 54 countries and documented in the FAO olive germplasm database (Bartolini 2008; Bracci et al., 2011; Acar, 2024b).

International efforts to document and conserve olive genetic resources have been ongoing for several decades. Within the RESGEN project coordinated by the International Olive Council, 139 olive varieties originating from 23 countries were catalogued, representing one of the earliest large-scale initiatives focused on the conservation and characterization of olive genetic resources (Bellini et al., 2008).

The International Olive Council (IOC) coordinates a global network of international and national olive germplasm banks to conserve olive biodiversity and support sustainable development, innovation, and resilience in the sector. The main objectives of this network include safeguarding olive genetic resources through the conservation of cultivars and wild genotypes in field collections, as well as their authentication and characterization using harmonized protocols. The network also facilitates the exchange of plant material under agreed standards and supports the development of documentation and certification tools such as the ***"World Catalogue of Olive Varieties and True Healthy Olive Cultivars (THOC)"*** (IOC, 2026). Beyond conserving diversity, ex situ collections provide plant material for breeding and research. Information on the identity and parentage of accessions helps improve the management of germplasm collections and supports the use of these resources in breeding programs (Gómez-Gálvez et al., 2026).

In addition, it promotes research and breeding activities related to climate resilience, disease resistance, and modern production systems.

Conservation strategies within this framework are based on both ex situ conservation, involving the maintenance of living trees in field collections such as Córdoba, Marrakech, and İzmir for the study of genetic traits, and in situ or on-farm conservation, which preserves olive diversity in traditional groves and landscapes, maintaining local adaptation, cultural heritage, and the ongoing evolution of cultivars (IOC, 2026).

The IOC currently recognizes eight international olive germplasm banks that collectively contribute to the conservation and study of olive genetic resources (Table 1). Research activities conducted within these institutions include genome-wide characterization of accessions, the development of core collections, and studies on traits associated with climate change adaptation and disease resistance (IOC, 2026).

Table 1. International Olive Germplasm Banks Recognized by the IOC

Country	Institution	Description
Spain	Córdoba (WOGBC, UCO/IFAPA)	A major global reference collection containing extensive accessions; also involved in molecular studies and capacity building activities
Morocco	INRA Tassaout	Maintains a large germplasm collection with emphasis on adaptation to dry and semi-arid environmental conditions
Türkiye	İzmir (WOGBC, ORI/TAGEM)	Provides representation of Eastern Mediterranean olive diversity across complementary agro-climatic environments
Argentina	San Juan (INTA)	Conserves Mediterranean and South American genetic material in Southern Hemisphere field conditions
Israel	Volcani Institute	Conducts research on stress tolerance and resistance to diseases in arid environments
Iran	Ministry of Agriculture Jihad (ARG)	Works on genetic characterization and breeding under severe continental climate conditions
Greece	ELGO DIMITRA (Crete)	Recently integrated international collection with strong representation of local and ancient cultivars
Italy	CREA OFA	Engaged in breeding programs and agronomic research within EU collaborative projects.

At the national level, olive germplasm collections established in several International Olive Council member countries, such as Albania, Algeria, Croatia, Cyprus, Egypt, France, Jordan, Lebanon, Libya, Montenegro, Portugal, the State of Palestine, Tunisia, and Uruguay, contribute to the conservation of regional olive diversity and wild olive genotypes. In addition to supporting local breeding and advisory programs, these repositories provide biological material and information for international characterization activities and IOC-related documentation resources (IOC, 2026).

Olive production and fruit quality in the Mediterranean basin are closely associated with climatic conditions. Recent projections indicate that the Mediterranean region is particularly vulnerable to climate change, mainly due to increasing temperatures and drought stress, which may adversely affect olive cultivation. Moreover, the adaptation of olive varieties depends not only on their genetic background but also on the environmental and pedoclimatic conditions of the cultivation area (Debbabi et al., 2022). Therefore, olive genetic resources represent a critical source of stress tolerance traits, and their effective utilization in pre-breeding programs will be increasingly important for developing resilient olive cultivars under future climate change scenarios.

PRE-BREEDING APPROACHES IN OLIVE

Olive trees exhibit a wide range of genetic diversity, making them a fundamental resource for breeding programs (Klepo et al., 2013; Montilon et al., 2022). Most of the currently cultivated olive varieties have been derived from clonal selection of superior trees identified through single-plant selection (Rallo et al., 2018). Olive breeding has traditionally focused on economically important traits such as fruit characteristics and oil content, and new varieties have been developed through cross-breeding programs; however, many of these have not achieved widespread commercial value (León et al., 2021).

In conventional olive breeding programs, the long juvenile period significantly delays the development of new cultivars (Bellini et al. 2008; Lavee et al. 1996; Atienza et al., 2014) and reduces the efficiency of selection, making the breeding process highly time-consuming. Moreover, although most olive cultivars originate from wild selections or are only a few generations removed from their wild ancestors, genetic diversity remains high even when using cultivated forms as parents. This high variability is also reflected in progeny

populations, making it necessary to evaluate large populations to identify individuals with specific target traits (Lavee, 2013). To overcome these limitations and increase breeding efficiency, a range of pre-breeding approaches have been developed. These approaches aim to facilitate the early identification of promising genotypes, improve the exploitation of genetic resources, and accelerate the development of new cultivars.

One of the major objectives of olive pre-breeding has been the reduction of the juvenile period through the identification of early selection criteria. Several studies have demonstrated that seedling vigor is closely associated with the onset of flowering. Pritsa et al. (2003) reported significant correlations between vegetative growth parameters, particularly seedling height and leaf traits, and flowering time. Similar findings were obtained by Kiran et al. (2008), showed that seedling height measured under greenhouse conditions was strongly associated with earlier reproductive development. Likewise, Rallo et al. (2008) found that vigor-related traits such as plant height and stem diameter were among the best predictors of juvenile period length. de la Rosa et al. (2006) further confirmed that seedling vigor traits especially plant height and stem diameter could be used for early preselection without negatively affecting important agronomic characteristics such as yield or fruit quality. In addition, Moreno-Alías et al. (2010) demonstrated that transplanting seedlings when they reached approximately 100 cm in height provided an optimal balance between practical field management and the induction of early flowering.

Beyond simple vigor measurements, architectural traits have also been investigated as indicators of juvenile period duration. Hammami et al. (2021) evaluated 794 one-year-old olive seedlings and reported that plant height, branch orientation, and other architectural characteristics were significantly associated with shorter juvenile periods. The authors demonstrated that combining vigor-related traits with architectural descriptors improved the efficiency of early seedling elimination compared with previous approaches, highlighting their value in pre-breeding programs.

Another important aspect of olive pre-breeding is the identification of traits associated with disease resistance. Cardoni et al. (2021) investigated the relationship between functional traits and tolerance to Verticillium wilt and reported substantial variation among cultivars. Tolerant genotypes were characterized by higher dry matter content, increased biomass allocation to

stems, specific root architectural patterns, and elevated root lignin concentrations. These findings suggest that functional trait variation may provide useful indirect indicators for early selection of resistant genotypes. Similarly, Arias-Calderón et al. (2015) screened wild and cultivated olive germplasm for resistance to Verticillium wilt and identified 13 resistant genotypes. The study highlighted the importance of wild olive populations as valuable sources for resistance breeding and proposed their use as rootstocks or parents in future breeding programs.

Pre-breeding strategies have also focused on improving the reliability of selection at intermediate stages of breeding. León et al. (2015) proposed the use of an intermediate evaluation stage based on vegetatively propagated replicates before multi-location field testing. Their results showed that fruit-related traits such as fruit size and oil content were relatively stable across evaluation stages, whereas vigor and crop load exhibited weaker consistency. The authors concluded that intermediate-stage evaluations can substantially improve selection accuracy and reduce the risk of advancing inferior genotypes.

Oil quality traits have likewise been explored as targets for early selection. de la Rosa et al. (2016) demonstrated considerable variability and moderate to high heritability for several olive oil quality parameters, including fatty acid composition and sterol content. The study suggested that some oil quality traits can be assessed relatively early in breeding programs, facilitating the identification of promising genotypes before extensive field evaluation.

Recent advances in molecular breeding have further strengthened olive pre-breeding efforts. Moret et al. (2025) validated molecular markers associated with fruit weight using a diverse panel of cultivated and wild olive genotypes. 16 of 40 candidate markers were confirmed, and 9 of them exhibited strong predictive ability for fruit weight variation. These validated markers provide valuable tools for marker-assisted selection and support the integration of genomic approaches into olive pre-breeding programs. In parallel, Rugini et al. (2016b) emphasized the importance of integrating molecular techniques, in vitro biotechnological techniques, including micropropagation, mutation approaches, and other biotechnological tools to overcome the limitations of conventional breeding and accelerate cultivar development.

Overall, these studies demonstrate that olive pre-breeding encompasses a wide range of strategies aimed at accelerating selection and improving

breeding efficiency. Early selection based on seedling vigor and architectural traits, screening for disease resistance, intermediate-stage evaluations, assessment of oil quality characteristics, and the integration of molecular markers have all contributed to reducing the time and resources required for cultivar development. A summary of the main pre-breeding approaches and their contributions to olive breeding programs is presented in Table 2.

Table 2. Major pre-breeding approaches reported in olive breeding

Reference	Pre-breeding approach	Target trait	Main findings
Pritsa et al. (2003)	Evaluation of early vegetative growth traits	Juvenile period / first flowering	Seedling height and leaf area were associated with the timing of first flowering.
de la Rosa et al. (2006)	Evaluation of vigor-related traits	Juvenile period / early selection	Plant height and stem diameter were associated with earlier flowering.
Kiran et al. (2008)	Assessment of seedling height as an early selection criterion	Juvenile period duration	Seedling height showed a strong relationship with juvenile period length.
Rallo et al. (2008)	Evaluation of vegetative seedling traits	Juvenile period / early selection	Plant height and stem diameter were associated with early flowering.
Moreno-Alías et al. (2010)	Assessment of transplant timing and seedling height	Juvenile period / early flowering	A seedling height of approximately 100 cm was identified as suitable for early selection.
Hammami et al. (2021)	Evaluation of architectural and vigor traits	Juvenile period and early selection	Plant height and architectural characteristics were associated with juvenile period duration.
León et al. (2015)	Evaluation of breeding selection stages	Selection efficiency and trait stability	A three-stage selection system was proposed; fruit and oil traits could be effectively selected at early stages.
de la Rosa et al. (2016)	Early evaluation of olive oil quality traits	Oil quality components	High variability and heritability were observed for several oil quality traits.
Atienza et al. (2014)	QTL analysis	Fruit and oil traits	QTL regions associated with important agronomic traits were identified for marker-assisted selection.
Arias-Calderón et al. (2015)	Controlled inoculation and resistance screening	Verticillium wilt resistance	13 resistant genotypes were identified.
Kaya et al. (2019)	GWAS and SNP marker analysis	Leaf and fruit traits	52 marker–trait associations were identified.

Reference	Pre-breeding approach	Target trait	Main findings
Cardoni et al. (2021)	Functional trait analysis	Verticillium wilt tolerance	Root architecture and lignin-related traits were associated with disease tolerance.
Moret et al. (2025)	Marker-assisted selection and machine learning	Fruit weight	16 of 40 candidate markers were validated, and nine showed high predictive power for fruit weight.

USE OF GENETIC RESOURCES IN OLIVE BREEDING

Olive is one of the oldest cultivated fruit tree species and includes a large number of local cultivars distributed throughout the Mediterranean Basin. The reasons leading to the high genetic variability of cultivated olive are mainly related to its allogamy and prevalent self-incompatibility, long cultivation history, and the persistence of ancient varieties over centuries (Kabassi et al.,2021; Schicchi et al., 2021; Baldoni et al., 2021). This diversity has provided breeders with a broad range of genetic resources that can be utilized for the improvement of agronomic traits and adaptation to different environmental conditions.

The conservation and characterization of olive genetic resources have become increasingly important for breeding programs. Wild olive populations are particularly valuable because they contain genetic variation that is often absent from cultivated germplasm. Díaz-Rueda et al. (2020) described the SILVOLIVE collection, which includes 146 wild olive genotypes representing six subspecies and early-generation hybrids. Molecular characterization confirmed the origin of the genotypes and revealed extensive genetic diversity, while phenotypic evaluations revealed substantial variation in vigor, branching architecture, and root-to-shoot allocation. The study further demonstrated the potential use of certain wild genotypes as rootstocks capable of modifying scion vigor, thereby expanding their breeding value beyond direct parental use.

Several studies have focused on molecular characterization of olive germplasm collections to improve the management and utilization of genetic resources. do Val et al. (2012) employed microsatellite markers to characterize Brazilian olive accessions and demonstrated the effectiveness of molecular tools for distinguishing genotypes and identifying genetically diverse breeding material. Similarly, Taranto et al. (2018) used genotyping-by-sequencing

technology to identify a large number of SNP markers across cultivated olive germplasm, revealing extensive genetic variation and generating genomic resources useful for future breeding applications.

The availability of high-density molecular markers has facilitated the application of genome-wide association studies (GWAS) in olive. Kaya et al. (2019) conducted one of the first large-scale GWAS analyses using accessions from Turkish Olive GenBank Resource and the USDA-ARS National Clonal Germplasm Repository. Significant marker-trait associations were identified for several economically important characteristics, including fruit weight, stone weight, fruit flesh-to-pit ratio, and leaf morphology. The identification of candidate genes linked to these traits provides valuable information for future marker-assisted breeding efforts.

Genetic resources also play a crucial role in understanding the pedigree structure and evolutionary history of olive cultivars. Gómez-Gálvez et al. (2026) performed a comprehensive parentage analysis involving more than 840 cultivars and demonstrated extensive genetic connectivity among Mediterranean olive varieties. The identification of key founder cultivars and reconstruction of pedigree relationships provide valuable information for germplasm management and parent selection, helping breeders maximize genetic diversity while avoiding excessive relatedness among breeding materials.

In addition to molecular characterization, phenotypic evaluation remains essential for the effective utilization of genetic resources. Belaj et al. (2020) assessed flowering and ripening phenology in cultivars maintained at the World Olive Germplasm Bank and identified substantial variation among genotypes. Such information is highly relevant for breeding programs aimed at developing cultivars adapted to changing climatic conditions and different production environments.

Broader assessments of olive breeding programs have repeatedly emphasized the importance of genetic resources for future cultivar development. Lavee (2013) highlighted that despite the extensive diversity present in both cultivated and wild olives, these resources remain underutilized in breeding programs. Likewise, Rallo et al. (2018) stressed that germplasm collections constitute the primary reservoir of variability for crossbreeding programs and that their effective use, combined with molecular tools and early

selection strategies, is essential for breeding progress. Debbabi et al. (2022) reached similar conclusions for Tunisian olive germplasm, emphasizing the need for better integration of local genetic resources into breeding programs to enhance productivity, oil quality, and climate resilience.

Overall, olive genetic resources encompass cultivated varieties, wild relatives, germplasm collections, and molecular diversity repositories that collectively provide the raw material for breeding. Their characterization through molecular, phenotypic, and genomic approaches is increasingly enabling breeders to identify valuable alleles, optimize parent selection, and develop cultivars better adapted to future production challenges.

Table 3. Major studies on the utilization of genetic resources in olive breeding

Reference	Genetic resource / approach	Objective	Main findings
do Val et al. (2012)	Microsatellite marker analysis of Brazilian olive accessions	Genetic diversity assessment and genotype identification	High genetic variation was detected and olive accessions were successfully differentiated.
Taranto et al. (2018)	GBS-based characterization of olive germplasm	Genome-wide diversity assessment	Extensive SNP variation was identified among cultivars, providing valuable genomic resources for breeding.
Díaz-Rueda et al. (2020)	Characterization of wild olive genetic resources (SILVOLIVE collection)	Genetic diversity assessment and rootstock evaluation	High genetic diversity and promising rootstock candidates were identified among wild olive genotypes.
Belaj et al. (2020)	Phenological evaluation of germplasm bank accessions	Assessment of flowering and ripening variation	Considerable phenological variability was observed among cultivars.
Gómez-Gálvez et al. (2026)	EST-SNP-based parentage analysis	Pedigree reconstruction and parent selection	Extensive pedigree relationships were identified, facilitating germplasm management and breeding decisions.

CHALLENGES AND FUTURE PERSPECTIVES

Olive breeding remains a slow and complex process due to the long juvenile phase of the species and the strong influence of environmental conditions on phenotypic expression. These factors complicate the evaluation of genotype performance and adaptability, often requiring multi-environment testing before reliable selection decisions can be made (Bellini et al., 2008). In addition, although early selection is necessary to reduce breeding costs and population size, it may result in the loss of potentially valuable genotypes, particularly for traits such as oil quality that can only be evaluated at later stages of development (León et al., 2015).

Climate change has become a major challenge for olive cultivation, particularly in Mediterranean regions where increasing temperatures, drought frequency, and reduced precipitation are expected to intensify in the coming decades. According to the Intergovernmental Panel on Climate Change (IPCC, 2021), global surface temperatures are projected to rise by around 2 °C by the end of the century, which will further exacerbate water scarcity and heat stress in agricultural systems. These environmental pressures, together with ongoing genetic erosion, threaten the long-term conservation of plant genetic resources and highlight the need for their effective preservation and sustainable use in breeding programs (Adıgüzel and Solmaz, 2023). In this context, pre-breeding serves as a critical link between germplasm conservation and cultivar development by facilitating the identification and transfer of useful alleles from cultivated varieties, landraces, and wild relatives into breeding populations (Rallo et al., 2018; Debbabi et al., 2022).

Future progress in olive breeding will depend on the integration of genetic resources with modern characterization and selection tools. Molecular markers provide efficient approaches for genotype identification and diversity assessment (do Val et al., 2012), while functional trait analyses may help identify genotypes with improved stress tolerance and adaptation potential (Cardoni et al., 2021). Together, these approaches can enhance the efficiency of pre-breeding programs and support the development of olive cultivars adapted to future environmental and production challenges.

Overall, the integration of genetic resource conservation, germplasm characterization, pre-breeding activities, strategies combining germplasm characterization, molecular tools, and early selection approaches will be

essential for increasing the efficiency of olive improvement programs and developing cultivars capable of meeting future production and environmental demands.

CONCLUSIONS

Olive genetic resources represent a fundamental source of variation for the long-term improvement of olive (*Olea europaea* L.) and play a central role in ensuring the sustainability of breeding programs. The species exhibits high genetic diversity derived from its long domestication history, the coexistence of wild, feral, and cultivated forms, and continuous natural and human-mediated selection. This diversity provides essential material for improving key agronomic traits such as yield stability, fruit and oil quality, and resistance to biotic and abiotic stresses. However, despite the richness of available germplasm, its effective use in breeding remains limited due to biological constraints including a long juvenile phase, high heterozygosity, and strong environmental effects on phenotypic expression, all of which slow down conventional breeding progress.

In this context, pre-breeding acts as a critical bridge between genetic resource conservation and cultivar development by enabling the identification, evaluation, and utilization of valuable alleles from wild relatives, local varieties, and underutilized germplasm. Early selection based on vigor, architectural traits, and functional characteristics, together with intermediate evaluation strategies, can significantly improve breeding efficiency and reduce generation time. At the same time, modern molecular tools such as high-throughput sequencing, genome-wide association studies, and marker-assisted selection have greatly improved the ability to characterize genetic resources and identify superior genotypes. When integrated with phenotypic and pedigree information, these approaches strengthen parent selection, enhance genetic gain, and support more efficient breeding decisions.

Despite these advances, challenges remain, particularly regarding the incomplete use of germplasm collections, the difficulty of evaluating complex traits under variable environments, and the need for stronger integration between conservation programs and active breeding pipelines. Given the increasing pressure of climate change, improving the exploitation of olive genetic resources has become even more urgent. Overall, future progress in

olive breeding will depend on a fully integrated strategy combining conservation, pre-breeding, and genomic technologies to develop cultivars that are more productive, resilient, and adaptable to changing environmental conditions.

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CHAPTER 20

THE EFFECT OF FAN PAD COOLING SYSTEMS ON INDOOR CLIMATE IN GREENHOUSES

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INTRODUCTION

Greenhouses are structures made of plastic or glass that provide a suitable microclimate for various crops and plants, protecting them from harsh and variable external weather conditions. During the day, intense solar radiation creates a greenhouse effect due to absorption, which makes the temperature inside the greenhouse higher than the ambient temperature. Consequently, plants and crops may dry out due to the high evaporation rate caused by excessive heat, which can lead to a decrease in production. It is therefore essential to use cooling technologies to provide a suitable growing environment (Xu et al., 2015). Various methods can be used to cool the environment inside a greenhouse, allowing plants to be grown under more suitable conditions. Natural ventilation is often considered the first step because it is cheaper and easier to operate, but this method is generally insufficient for releasing excess energy inside during sunny summer days (Baille, 1999). One of the most effective solutions for mitigating climate conditions inside greenhouses is the use of evaporative cooling systems. The basic principle of this system is the conversion of sensible heat into latent heat. For this purpose, water is supplied to the greenhouse environment directly by misting or rain showers, or with wet pads. Evaporative cooling simultaneously reduces the temperature and vapor pressure deficit, ensuring that the greenhouse interior temperature is lower than the outside air temperature (Montero et al., 1990; Arbel et al., 1999).

In greenhouses in hot regions, temperatures can easily exceed 38ºC if there is no cooling system. High temperatures reduce plant quality and worker productivity. Evaporative cooling is the most commonly used method for reducing greenhouse internal temperatures. In regions with cooler climates, temperatures rise to 30-33ºC during the summer months, and greenhouse internal temperatures increase even further. Summer temperatures in cool climate regions are lower than in hot climate regions. Therefore, by cooling greenhouses by 10-12ºC with a fan pad system, the greenhouse temperature can be lowered below the outside air temperature, allowing production to continue in greenhouses even in summer (Atılgan and Öz, 2007). In a study by Kittas et al. (2003), a fan pad system was used to cool a greenhouse while maintaining an internal temperature of 28°C. The system was found to be 80% efficient, achieving a temperature reduction 10°C lower than the outside temperature. The researchers also determined that humidity content was important in

determining the cooling efficiency of the fan pad system. Additionally, the researchers noted that high performance was achieved with the fan pad system at low humidity levels. In a study investigating the effectiveness of a fan pad system in a greenhouse where tomatoes, peppers and cucumbers were grown (Daives, 2005), it was stated that the indoor temperature of the greenhouse was cooled by 15°C compared to the outdoor temperature when using the fan pad system. It was also stated that this system had a cooling efficiency that was 5°C better than that of other traditional cooling systems, and that the growing season of plants such as tomatoes and cucumbers could be extended to between seven and 12 months.

This study evaluated the effect of fan pad cooling systems on the indoor climate in greenhouses. For this purpose, the temperature of the air passing through the pad, the changes in temperature and relative humidity inside the greenhouse, and the amount of water consumed were calculated at different air change rates and pad efficiencies. Based on the calculations obtained, some measures to improve the usability and efficiency of evaporative cooling systems in regions where greenhouses will be built are suggested.

DETERMINING THE ACHIEVABLE INDOOR CLIMATE VALUES FOR FAN PAD COOLING

The success of evaporative cooling methods depends on accurate design. Therefore, knowing the ambient temperature and relative humidity of the region where evaporative cooling will be applied is crucial for system effectiveness. For this reason, determining the achievable temperature and relative humidity values indoor the greenhouse beforehand is important for the system's feasibility. The effect of an evaporative cooling system on the indoor climate in a greenhouse was calculated using the equations given below (Baytorun and Makauy, 2019).

The number of air changes per unit area is determined by Equation 1.

$$V_A = \frac{q_L}{\rho_i * (h_i - h_o)} \quad (1)$$

In the equation; V_A is ventilation rate (m^3/m^2 h), q_L is the amount of heat to be removed from the greenhouse (W/m^2), ρ is density of air (kg/m^3)

In evaporative cooling, the temperature to be reached at the outlet of the pads is calculated from Equation 2.

$$\theta_L^* = \theta - \eta \times (\theta - \theta_{FK}) \quad (2)$$

In the equation; θ^*_L is dry bulb temperature at pad outlet (°C), θ is the dry bulb temperature (°C), η is pad efficiency (%), θ_{FK} is wet bulb temperature (°C).

The temperature reached based on the final enthalpy value and moisture content of the air in the greenhouse is determined by Equation 3.

$$\theta_i = \frac{h_i - x_i * r_o}{c_{pL} + c_{pw} * x_i} \quad (3)$$

In the equation; h_i is enthalpy value of the air entering the greenhouse at constant enthalpy value, x_i is moisture content of the air entering the greenhouse after being humidified at constant enthalpy value (kJ/kg), r_o is heat of evaporation (kJ/kg), c_{pL} is specific heat of air (kJ/kg °C), c_{pw} is specific heat of water vapor (kJ/kg °C)

The new relative humidity of the air passing through the cooling pad was determined using Equation 4.

$$\varphi_i = \frac{P * x_i}{(0.622 + x_i) * p_{ws}} \quad (4)$$

In the equation; φi is new relative humidity (%), P is atmospheric pressure (kPa), p_{ws} the saturation vapor pressure (kPa)

The consumption of water for fan pad cooling was calculated with Equation 5.

$$m_w = V_A * \rho * (x_i - x_o) \quad (5)$$

In the equation; m_w is amount of water required (kg/m^2 h), ρ is density of air (kg/m^3), x_o is moisture content (%)

THE EFFECT OF FAN PAD COOLING ON THE INDOOR CLIMATE OF A GREENHOUSE

Calculations were made for greenhouses under conditions where the outdoor radiation value is 900 W/m^2, the air exchange rate is 125 m^3/m^2 h, and the pad efficiency is 80%. Accordingly, the temperature to be reached in front of the pad, the indoor temperature and relative humidity values, and the amount of water consumed are given in Table 1.

Table 1. Changes in the indoor climate when the air exchange rate in the greenhouse is 125 m^3/m^2 h and the pad efficiency is 80%.

T_{mean} (°C)	RH_{mean} (%)	θ^*_L (°C)	No shading		Shading rate 50%		m_w (kg/m^2 h)
			θ_i (°C)	φ_i (%)	θ_i (°C)	φ_i (%)	
30.0	30.0	19.8	29.2	45.4	24.5	59.8	0.54
30.0	35.0	20.8	30.2	46.6	25.5	61.3	0.48
30.0	40.0	21.7	31.1	47.8	26.4	62.8	0.44
30.0	45.0	22.5	31.9	49.2	27.2	64.5	0.39
30.0	50.0	23.3	32.8	50.3	28.0	65.9	0.35
30.0	55.0	24.1	33.6	51.2	28.8	67.1	0.31
30.0	60.0	24.8	34.3	52.4	29.6	68.5	0.27
30.0	65.0	25.5	35.1	53.3	30.3	69.7	0.23
30.0	70.0	26.2	35.8	54.1	31.0	70.6	0.19
30.0	75.0	26.9	36.5	55.1	31.7	71.9	0.16

T_{mean} is outdoor mean temperature, RH_{mean} is outdoor relative humidity, θ^*_L is dry bulb temperature at pad outlet, θ_i is indoor temperature, φ_i is indoor relative humidity, m_w is amount of water required

Table 1 shows that when the outside temperature is 30°C and the relative humidity is 30%, the air temperature in front of the pad is 19.8°C. When the outside relative humidity is 75%, the temperature in front of the pad is calculated as 26.9°C. At the same temperature, increasing the outside relative humidity from 30% to 75% increased the temperature of the air passing through the pad by 7.0°C. The study conducted by Boyacı and Atılgan (2025) found that the average cooling effect was between 5.4 and 7.9°C. Vala et al. (2016) found an average cooling effect of 8.0°C. Similarly, these values are compatible with the calculated values. Therefore, increasing relative humidity values caused a decrease in the temperature in front of the pad.

Calculations were made for greenhouses under conditions where the outdoor radiation value is 900 W/m^2, the air exchange rate is 250 m^3/m^2 h, and the pad efficiency is 80%. Accordingly, the temperature to be reached in front of the pad, the indoor temperature and relative humidity values, and the amount of water consumed are given in Table 2.

Table 2. Changes in the indoor climate when the air exchange rate in the greenhouse is 250 m^3/m^2 h and the pad efficiency is 80%.

T_{mean} (°C)	RH_{mean} (%)	θ^*_L (°C)	No shading		Shading rate 50%		m_w (kg/m^2 h)
			θ_i (°C)	φ_i (%)	θ_i (°C)	φ_i (%)	
30.0	30.0	17.3	24.5	59.8	22.2	68.9	1.07
30.0	35.0	18.5	25.5	61.3	23.2	70.6	0.97
30.0	40.0	19.6	26.4	62.8	24.0	72.3	0.87
30.0	45.0	20.6	27.20	64.5	24.8	74.2	0.79
30.0	50.0	21.6	28.0	65.9	25.7	75.8	0.70
30.0	55.0	22.6	28.8	67.1	26.5	77.1	0.62
30.0	60.0	23.5	29.6	68.5	27.2	78.7	0.54
30.0	65.0	24.4	30.3	69.7	27.9	79.9	0.46
30.0	70.0	25.3	31.0	70.7	28.6	81.1	0.39
30.0	75.0	26.1	31.7	71.9	29.3	82.4	0.32

T_{mean} is outdoor mean temperature, RH_{mean} is outdoor relative humidity, θ^*_L is dry bulb temperature at pad outlet, θ_i is indoor temperature, φ_i is indoor relative humidity, m_w is amount of water required

Table 2 shows that when the outside temperature is 30°C and the relative humidity is 30%, the indoor temperature is 24.5°C. When the outside relative humidity is 75%, the indoor temperature is calculated to be 31.7°C. With 50% shading in the greenhouse, the indoor temperature is 22.2°C, while with 75% relative humidity it is calculated to be 29.3°C.

Tables 1 and 2 show that at 80% pad efficiency, increasing the air exchange rate from 125 m^3/m^2 h to 250 m^3/m^2 h resulted in a decrease in pad front temperatures. Accordingly, while the pad front temperature was 19.8°C with 30% ambient relative humidity, it decreased to 17.3°C due to the increased air exchange rate. In the absence of shading nets, the indoor temperature decreased from 29.2°C to 24.5°C. With the use of shading nets, it decreased from 24.5°C to 22.2°C. Heat stress is caused to plants by high solar intensity, so shading net systems are used in greenhouses (Holcman et al., 2012). Therefore, increasing the ventilation rate and using shading nets contributed to reducing indoor temperatures.

Plants grown in greenhouses have adapted to temperatures of 17-27°C (Baytorun and Makauy, 2019). To maintain the optimum indoor temperature of 27°C, an air exchange rate of 125 m^3/m^2 h, an external relative humidity of 45%, and the use of shading nets are required. Even with an external relative humidity of 60%, using shading nets and an air exchange rate of 250 m^3/m^2 h will still allow the indoor temperature to be maintained at suitable levels.

Table 1 shows that when the outdoor relative humidity is 30%, the amount of water consumed will be 0.54 kg/m^2 h. Table 2 shows that when the outdoor relative humidity is 75%, the amount of water consumed will be 1.07 kg/m^2 h. Therefore, lower relative humidity levels mean that the dry air causes more water to evaporate. This means that more water is consumed per unit area. In the study conducted by Boyacı and Atılgan (2025) found that the average water consumption in fan pad cooling systems was 0.9-1.0 kg/m^2 h. It can be seen that the obtained values are similar.

Calculations were made for greenhouses under conditions where the outdoor radiation value is 900 W/m^2, the air exchange rate is 125 m^3/m^2 h, and the pad efficiency is 90%. Accordingly, the temperature to be reached in front of the pad, the indoor temperature and relative humidity values, and the amount of water consumed are given in Table 3.

Table 3. Changes in the indoor climate when the air exchange rate in the greenhouse is 125 m^3/m^2 h and the pad efficiency is 90%.

T_{mean} (°C)	RH_{mean} (%)	θ^*_L (°C)	No shading		Shading rate 50%		m_w (kg/m² h)
			θ_i (°C)	φ_i (%)	θ_i (°C)	φ_i (%)	
30.0	30.0	17.3	27.9	50.9	23.2	67.2	0.61
30.0	35.0	18.5	29.0	51.5	24.3	67.8	0.55
30.0	40.0	19.6	30.0	52.2	25.3	68.7	0.49
30.0	45.0	20.6	30.9	53.1	26.3	69.8	0.44
30.0	50.0	21.6	31.9	53.8	27.2	70.6	0.40
30.0	55.0	22.6	32.8	54.3	28.1	71.2	0.35
30.0	60.0	23.5	33.6	55.0	28.9	72.1	0.30
30.0	65.0	24.4	34.5	55.6	29.7	72.7	0.26
30.0	70.0	25.3	35.3	56.0	30.5	73.2	0.22
30.0	75.0	26.1	36.1	56.7	31.3	73.9	0.18

T_{mean} is outdoor mean temperature, RH_{mean} is outdoor relative humidity, θ^*_L is dry bulb temperature at pad outlet, θ_i is indoor temperature, φ_i is indoor relative humidity, m_w is amount of water required

Table 3 shows that if no shading is applied in the greenhouse, the relative humidity inside the greenhouse will be 50.9% when the outside relative humidity is 30%, and 56.7% when the outside relative humidity is 75%. If 50% shading is applied, the relative humidity inside the greenhouse will be 67.2% when the outside relative humidity is 30%, and 73.9% when the outside relative humidity is 75%. Greenhouses are used in many countries around the world, and cooling inside the greenhouses is essential to increase production during hot summer months. Evaporative systems were developed to cool greenhouses during summer temperatures, thus facilitating plant growth. However, evaporative systems not only reduce temperature but also increase relative humidity (Montero et al., 1990). The cooling effectiveness of evaporative cooling systems depends on the relative humidity of the outdoor environment. Thus, the higher the outdoor relative humidity, the less effective the fan-pad system will be (Kumar et al., 2009). Therefore, at lower relative humidity levels, the drier air will cause more water to evaporate, increasing the relative humidity inside the greenhouse.

Calculations were made for greenhouses under conditions where the outdoor radiation value is 900 W/m^2, the air exchange rate is 250 m^3/m^2 h, and the pad efficiency is 90%. Accordingly, the temperature to be reached in front of the pad, the indoor temperature and relative humidity values, and the amount of water consumed are given in Table 1.

Tablo 4. Changes in the indoor climate when the air exchange rate in the greenhouse is 250 m^3/m^2 h and the pad efficiency is 90%.

T_{mean} (°C)	RH_{mean} (%)	θ^*_L (°C)	No shading		Shading rate 50%		m_w (kg/m^2 h)
			θ_i (°C)	φ_i (%)	θ_i (°C)	φ_i (%)	
30.0	30.0	18.6	23.2	67.2	20.9	77.5	1.21
30.0	35.0	19.7	24.3	67.8	21.9	78.2	1.09
30.0	40.0	20.6	25.3	68.7	22.9	79.1	0.99
30.0	45.0	21.5	26.3	69.8	23.9	80.3	0.89
30.0	50.0	22.4	27.2	70.6	24.8	81.2	0.79
30.0	55.0	23.3	28.1	71.2	25.7	81.8	0.70
30.0	60.0	24.2	28.9	72.1	26.5	82.8	0.61
30.0	65.0	25.0	29.7	72.7	27.3	83.5	0.52
30.0	70.0	25.8	30.5	73.2	28.2	83.9	0.44
30.0	75.0	26.5	31.3	73.9	28.9	84.9	0.36

T_{mean} is outdoor mean temperature, RH_{mean} is outdoor relative humidity, θ^*_L is dry bulb temperature at pad outlet, θ_i is indoor temperature, φ_i is indoor relative humidity, m_w is amount of water required

Tables 3 and 4 show that at 90% ventilation efficiency, increasing the air exchange rate from 125 m^3/m^2 h to 250 m^3/m^2 h resulted in an increase in relative humidity. Accordingly, with an outdoor relative humidity of 30% and no shading net, the relative humidity increased from 50.9% to 67.2%. With the use of shading net, it increased from 67.2% to 77.5%. Therefore, increasing the ventilation rate and using shading net contributed to the increase in indoor relative humidity. Shivpuje et al. (2018) found that the mean relative humidity of the air entering the cooling pad was between 42.1% and 48.2%, while the relative humidity of the air exiting the pad was between 65.4% and 78.7%. The results obtained were found to be compatible with the study.

In order to maintain an indoor temperature of 27°C, an air exchange rate of 125 m^3/m^2 h and an external relative humidity of 50% are required, as well as the use of shading nets. Even with an external relative humidity of 65%, using shading nets and an air exchange rate of 250 m^3/m^2 h will still allow the indoor temperature to be maintained at suitable levels.

Table 3 shows that when the outdoor relative humidity is 30%, the amount of water consumed will be 0.61 kg/m^2 h. Table 4 shows that when the outdoor relative humidity is 75%, the amount of water consumed will be 1.21 kg/m^2 h. Therefore, low outdoor relative humidity and increased air exchange rates mean lower indoor temperatures and higher relative humidity. Furthermore, this means that more water is consumed per unit floor area.

CONCLUSION AND RECOMMENDATIONS

Evaporative cooling methods, used during periods when high temperatures make plant cultivation impossible, can make the greenhouse environment suitable for plant cultivation. However, since the success of these methods depends on external climatic conditions, accurately determining the climate of the region where the application will be carried out is of great importance. For this purpose, the temperature and relative humidity values to be achieved in the interior must be suitable for plant cultivation. Otherwise, low system performance will not bring the interior environment to the desired temperature and relative humidity values. This situation will cause the profitability of the investment to be questioned. For this purpose, it is necessary to determine the air change rates taking into account the greenhouse volume. Appropriate air change rates will increase air-water contact in the air passing

through the pad, reducing the temperature inside while increasing the relative humidity. To increase the efficiency of the system, greenhouse openings must be checked and their airtightness ensured.

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CHAPTER 21

HYPERSPECTRAL IMAGE ANALYSIS IN PRECISION AGRICULTURE

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INTRODUCTION

The rising demand for agricultural production, coupled with growing concerns regarding resource use efficiency and environmental sustainability, has accelerated the development of data-driven crop management strategies. Precision agriculture is based on the acquisition, processing, and interpretation of spatially and temporally variable information to support site-specific management decisions. In this context, imaging and sensing technologies have become vital tools for gathering information on crop status, plant development, and environmental interactions through non-destructive methods. Among these technologies, hyperspectral imaging (HSI) has attracted particular interest due to its capacity to simultaneously provide detailed spectral and spatial information and to reveal subtle changes that might go undetected by conventional imaging systems (Lu et al., 2020; Sethy et al., 2022; Ram et al., 2024).

Hyperspectral imaging combines traditional digital imaging with spectroscopy by recording reflectance data across numerous narrow, contiguous wavelength bands. Consequently, a hyperspectral image is represented as a three-dimensional data cube comprising two spatial dimensions and one spectral dimension. Depending on the sensor configuration, the spectral information can span the visible, near-infrared, and shortwave infrared regions of the electromagnetic spectrum. The interaction of incident radiation with plant tissues is influenced by various structural, physiological, and biochemical properties, including pigment composition, cellular organization, leaf water content, and chemical constituents. These interactions give rise to characteristic spectral responses that can be utilized to examine plant traits and crop status non-destructively (Lu et al., 2020; Sarić et al., 2022; Zhang et al., 2024). Thus, this combination of spectral sensitivity and spatial positioning distinguishes hyperspectral imaging from traditional spectroscopy, where spectral measurements are typically obtained without detailed spatial information.

The spectral response of vegetation varies depending on physiological processes and changes in plant tissue. While alterations in chlorophyll and other photosynthetic pigments primarily affect reflectance in the visible region, the internal structure of the leaf exerts a strong influence on near-infrared reflectance. Water content and various biochemical constituents can also be

linked to specific absorption characteristics at particular wavelengths. These properties have enabled the application of hyperspectral imaging to a wide range of precision agriculture challenges, including crop disease detection, the identification of biotic and abiotic stresses, nutrient status assessment, the estimation of biochemical and biophysical traits, plant phenotyping, crop classification, and the evaluation of crop quality (Sarić et al., 2022; Avola et al., 2023; García-Vera et al., 2024; Ram et al., 2024). Since physiological changes can alter spectral responses before visible symptoms appear, hyperspectral data are also attracting significant interest for the early assessment of plant stress and disease conditions (García-Vera et al., 2024; Ali et al., 2024).

However, the richness of hyperspectral information also presents significant analytical challenges. Hundreds of adjacent spectral bands may contain highly correlated or redundant information; meanwhile, sensor noise, scattering effects, illumination variability, background interference, and variations in plant geometry can influence the recorded spectral response. The high dimensionality of hyperspectral data further increases computational requirements and can compromise model robustness when the number of variables exceeds the number of observations. Consequently, raw hyperspectral images typically require a series of processing steps to derive meaningful agricultural information. These steps encompass radiometric calibration, noise reduction, spectral preprocessing, image segmentation, region-of-interest selection, dimensionality reduction, feature extraction, and wavelength selection (Lu et al., 2020; Sethy et al., 2022; Ram et al., 2024). The selection and sequencing of these processing steps are of critical importance, as they can significantly impact both the spectral information preserved in the dataset and the performance of subsequent analytical models.

Traditional analysis of agricultural hyperspectral data has relied heavily on statistical and chemometric approaches, including principal component analysis, partial least squares regression, discriminant analysis, and other multivariate techniques. Subsequently, machine learning methods such as support vector machines, random forests, and artificial neural networks offered more flexible approaches for modeling non-linear relationships within high-dimensional spectral data. More recently, advancements in deep learning have shifted hyperspectral image analysis from a primary focus on spectral modeling

toward integrated spectral-spatial analysis. Convolutional architectures and associated learning approaches are capable of utilizing not only wavelength-dependent information but also spatial patterns associated with plant tissues, symptoms, and structural features (Ali et al., 2024; García-Vera et al., 2024; Zhang et al., 2024). However, improvements in prediction accuracy do not eliminate fundamental issues regarding model interpretability, dataset size, transferability across varieties and environments, and the reproducibility of hyperspectral processing workflows.

Therefore, the effective use of hyperspectral imaging in precision agriculture depends not only on sensor performance but also on the methodological framework employed to transform hyperspectral data into agriculturally meaningful information. A clear understanding of the processes including image acquisition, preprocessing, segmentation, feature extraction, dimensionality reduction, and modelling is crucial for interpreting hyperspectral responses and assessing the reliability of the resulting crop information. This chapter presents the fundamental principles of hyperspectral imaging and focuses on the primary image processing and analysis approaches used in precision agriculture. Particular emphasis is placed on hyperspectral image preprocessing, spectral and spatial feature extraction, dimensionality reduction, and statistical and machine learning-based modeling processes, as well as their applications in plant disease detection, stress assessment, nutrient and pigment estimation, phenotyping, biomass and growth analysis, and agricultural product quality assessment. Furthermore, current limitations and emerging trends in hyperspectral image analysis are discussed.

1. FUNDAMENTALS OF HYPERSPECTRAL IMAGING

1.1. Spectral Characteristics of Vegetation

The interpretation of hyperspectral images in precision agriculture requires a fundamental understanding of the interaction between electromagnetic radiation and plant tissues. When incident radiation reaches a leaf surface, it may be absorbed, reflected, or transmitted, depending on the wavelength and the optical properties of the tissue. These optical properties are determined by various factors, such as pigment concentration, water content, biochemical composition, surface characteristics, and the leaf's internal anatomical structure. Consequently, the spectral reflectance of vegetation is not

arbitrary; rather, it represents the combined effects of the plant's multiple biochemical and structural characteristics (Jacquemoud & Baret, 1990; Peñuelas & Filella, 1998; Wong, 2023).

In the visible region of the electromagnetic spectrum, approximately the 400–700 nm, range the reflectance properties of vegetation are primarily determined by photosynthetic pigments. Chlorophyll a and chlorophyll b strongly absorb radiation in the blue and red regions, resulting in relatively low reflectance at these wavelengths. In contrast, absorption is weaker in the green region, giving rise to the characteristic green appearance of healthy vegetation. Carotenoids and other accessory pigments also contribute to absorption patterns, particularly in the blue and blue-green regions. Consequently, variations in pigment concentration alter the shape and magnitude of the visible reflectance spectrum; these changes provide a basis for the non-destructive estimation of chlorophyll and other pigments critical to photosynthesis (Blackburn, 2007; Karabourniotis et al., 2021).

One of the most diagnostically significant regions in vegetation spectroscopy is the transition zone between red and near-infrared wavelengths, commonly referred to as the "red-edge" region. This region is characterized by a rapid increase in reflectance, shifting from the low reflectance values caused by strong chlorophyll absorption at red wavelengths to the high reflectance values observed in the near-infrared region. The position and slope of the red edge are sensitive to changes in chlorophyll concentration, physiological status, and, in some instances, plant stress. Consequently, red-edge characteristics have been extensively studied as indicators of vegetation condition; furthermore, these features hold particular importance in hyperspectral analysis. This is because narrow, contiguous spectral bands allow for the much more precise detection of subtle variations in this region compared to broadband imaging systems (Peñuelas & Filella, 1998; Blackburn, 2007; Lu et al., 2020).

Beyond the red-edge region, vegetation typically exhibits high reflectance in the near-infrared (NIR) region. Unlike the visible region, where spectral response is determined by pigment absorption, near-infrared reflectance is largely influenced by the leaf's internal structure. Interfaces between cell walls, intercellular air spaces, and water-containing tissues lead to multiple scattering of radiation, resulting in the formation of a characteristic,

highly reflective plateau. Consequently, changes in leaf anatomy, tissue organization, thickness, senescence, and structural damage can alter near-infrared reflectance, even if pigment concentrations remain relatively constant (Jacquemoud & Baret, 1990; Karabourniotis et al., 2021; Wong, 2023). This structural sensitivity is particularly important in agricultural applications, as many stress responses entail simultaneous changes in both biochemical composition and tissue organization.

Water-related absorption features appear in both the near-infrared and shortwave infrared regions; distinct features occur around 970 and 1200 nm, while stronger absorption bands are found near 1450 and 1940 nm. The depth and shape of these absorption features can change as plant water content varies, providing spectral information regarding the plant's water status. The shortwave infrared region also contains absorption features associated with biochemical constituents such as protein, cellulose, lignin, starch, and other organic compounds; however, these signals can overlap and are generally more difficult to interpret directly compared to the dominant pigment-related features in the visible region (Jacquemoud & Baret, 1990; Lu et al., 2020; Wong, 2023). These characteristic reflectance patterns of healthy vegetation across the visible, red-edge, near-infrared, and shortwave infrared regions are illustrated in **Figure 1**.

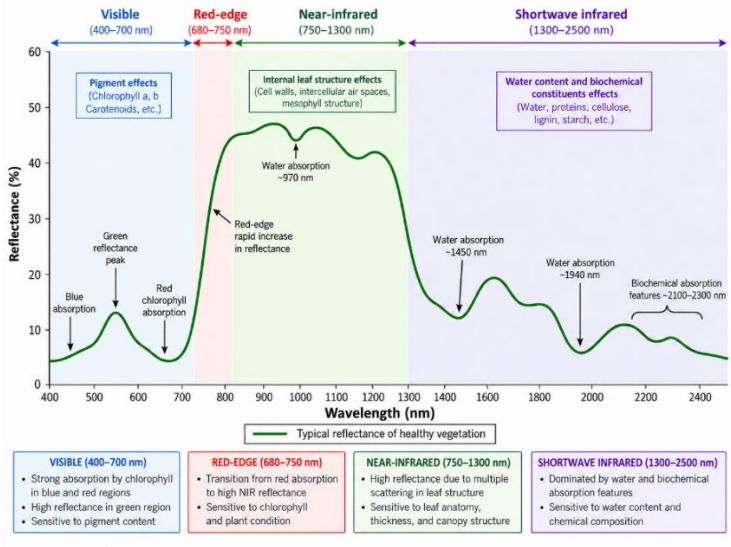

Figure 1. Schematic representation of the typical spectral reflectance of healthy vegetation (Peñuelas & Filella, 1998; Blackburn, 2007; Lu et al., 2020).

Therefore, the spectral response of vegetation should be treated not as the response of a single biochemical component, but as an integrated expression of numerous plant traits. Changes in reflectance at a specific wavelength may be associated with pigment degradation, water loss, structural alterations, biochemical changes, or combinations of these processes.

Furthermore, spectral responses observed at the leaf scale may differ from those obtained from whole plants or vegetation layers (canopy), as factors such as canopy architecture, leaf angle distribution, shadows, soil background, and multiple scattering introduce additional sources of variability. These scale-dependent effects are of particular importance when interpreting hyperspectral data in the context of precision agriculture, where the objective is often to relate spectral variations to physiological or agronomic traits under heterogeneous growing conditions (Peñuelas & Filella, 1998; Lu et al., 2020; Wong, 2023).

Therefore, hyperspectral imaging offers a significant advantage over conventional color imaging and broadband spectral measurements. The availability of narrow, contiguous wavelength bands enables the detailed characterization of absorption features, reflectance gradients, red-edge behavior, and subtle spectral differences associated with plant biochemical and structural properties. However, the vast amount of spectral information also increases redundancy and complicates direct biological interpretation. Consequently, extracting meaningful vegetation information requires appropriate preprocessing, wavelength selection, feature extraction, and modeling strategies, which are addressed in the subsequent sections of this chapter.

1.2. Hyperspectral Data Cube and Spectral–Spatial Information

Hyperspectral imaging differs fundamentally from traditional spectroscopy because it acquires spectral information alongside spatial information. A hyperspectral image is typically represented as a three-dimensional data structure, commonly referred to as a hyperspectral data cube or hypercube. While two dimensions of this cube correspond to the spatial coordinates (x and y) of the scene, the third dimension represents the wavelength (λ). Consequently, each spatial location within the hyperspectral image contains a spectral vector composed of reflectance or radiance values

measured across numerous narrow and contiguous wavelength bands (Plaza et al., 2009; Lu et al., 2020). The three-dimensional organization of hyperspectral data comprising two spatial dimensions and one spectral dimension is illustrated schematically in **Figure 2**.

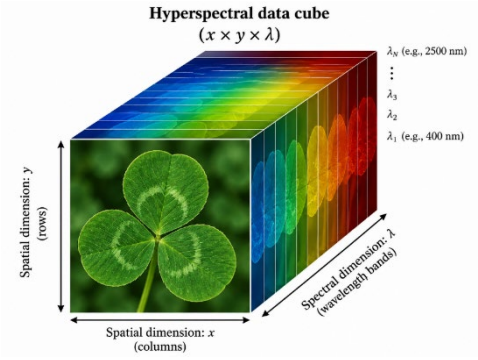

Figure 2. Schematic representation of a hyperspectral data cube (Lu et al., 2020; Ram et al., 2024).

This structure enables the interpretation of hyperspectral data from two complementary perspectives. From a spatial perspective, a specific wavelength band can be visualized as a two-dimensional grayscale image showing the spatial distribution of reflectance values at that wavelength; consequently, different wavelengths can highlight distinct plant structures or biochemical properties. From a spectral perspective, selecting a single pixel yields a reflectance spectrum that characterizes the wavelength-dependent optical response of the material or plant tissue in question. Thus, hyperspectral imaging combines the capabilities of digital imaging which provides information on location, shape, and spatial arrangement with those of spectroscopy, which offers insights into material composition and physicochemical properties (Manolakis et al., 2003; Gowen et al., 2007; Lu et al., 2020).

Depending on sensor configuration and spectral range, the spectral dimension of a hyperspectral cube typically comprises tens to hundreds of narrow bands. Since vegetation reflectance varies continuously with wavelength, there is often high correlation between adjacent bands. While this spectral continuity enables the identification of subtle absorption features and minor variations in spectral shape, it also leads to significant data redundancy within the dataset. Consequently, hyperspectral images are characterized by

high dimensionality and large data volumes; this can increase computational requirements and complicate subsequent statistical analysis and modeling processes (Plaza et al., 2009; Bioucas-Dias et al., 2013).

A significant advantage of hyperspectral imaging is the ability to analyze spatial and spectral domains either independently or in conjunction. Pixel-based spectral analysis treats each pixel as a distinct spectrum, primarily leveraging differences in wavelength-dependent reflectance patterns. This approach is widely used for estimating biochemical properties or distinguishing plant conditions. However, hyperspectral images also contain information regarding the spatial arrangement of neighboring pixels such as texture, shape, boundaries, symptom distribution, and textural heterogeneity. Consequently, integrating these spatial features with spectral information can provide a more comprehensive representation of plant status compared to analyses based solely on spectral signatures (Plaza et al., 2009; Li et al., 2019).

The concept of spectral-spatial information is particularly important in agricultural image analysis because plant tissues are rarely spatially homogeneous. A single leaf may simultaneously contain healthy tissue, veins, necrotic zones, chlorotic areas, shadows, and background pixels, each producing distinct spectral responses. Consequently, averaging all pixels within an image or region of interest can obscure locally significant diagnostic variations. Conversely, pixel-level analysis preserves local spectral differences but may also increase susceptibility to noise and intra-class variability. Therefore, appropriate segmentation, region-of-interest definition, and spectral-spatial feature extraction are fundamental steps for deriving representative information from hyperspectral plant images.

The high information density of the hyperspectral cube is both a fundamental advantage and a primary analytical challenge. When hundreds of spectral variables are combined with potentially millions of spatial pixels, they can generate datasets far larger than those produced by conventional RGB imaging. Consequently, effective hyperspectral image analysis requires methods capable of reducing redundancy while preserving relevant spectral and spatial information. Dimensionality reduction, wavelength selection, image segmentation, feature extraction, and spectral-spatial modeling are among the key approaches used to address these challenges and will be discussed in subsequent sections.

1.3. Hyperspectral Image Acquisition Systems

Hyperspectral images can be acquired using various sensor architectures that differ in how spatial and spectral information is recorded. Common configurations include point-scanning, line-scanning (push-broom), wavelength-scanning, and snapshot systems. Among these, line-scanning and snapshot sensors stand out particularly in agricultural imaging because they provide both spatial and spectral information regarding plant surfaces (Lu et al., 2020). In line-scanning systems, a full spectral profile is recorded along a single spatial line, while the second spatial dimension is generated through the relative motion between the sensor and the target. Although this configuration typically offers high spectral resolution and efficient spectral sampling, image quality can be affected by motion instability and geometric distortions during data acquisition (Ram et al., 2024).

In contrast, snapshot hyperspectral systems acquire spatial and spectral information in a single exposure or within a very short data acquisition interval. The primary advantage of these systems is that they do not require mechanical scanning, making them suitable for dynamic scenes and applications requiring rapid image acquisition. However, this advantage often entails trade-offs regarding factors such as spatial resolution, spectral resolution, spectral range, or the number of available bands. While wavelength-scanning systems sequentially acquire two-dimensional images at different wavelengths, point-scanning systems collect spectra sequentially at discrete spatial locations and require scanning across both spatial dimensions to construct a complete hyperspectral image (Lu et al., 2020; Ram et al., 2024).

The choice of an imaging system depends on the intended application, the required spectral and spatial resolution, the imaging speed, the lighting conditions, and the degree of scene stability. In controlled laboratory settings, scanning systems are generally preferred because uniform illumination and controlled movement are more easily achieved. For field or dynamic measurements, faster imaging architectures can mitigate motion-induced artifacts and temporal inconsistencies. Regardless of the imaging configuration, stable illumination, proper sensor calibration, and consistent imaging geometry are fundamental to obtaining reliable hyperspectral data suitable for subsequent image analysis.

2. HYPERSPECTRAL IMAGE PREPROCESSING

Hyperspectral images typically require preprocessing to ensure that spectral information can be reliably interpreted or utilized in statistical modeling. Raw data may contain variations arising from sensor response, illumination conditions, sample geometry, background materials, and random noise. If these effects are not adequately controlled, they can mask biologically significant spectral changes and compromise the robustness of subsequent classification or regression models. Consequently, the preprocessing workflow in agricultural hyperspectral imaging typically encompasses radiometric calibration, spectral correction, noise reduction, and the separation of the target object from the background (Lu et al., 2020; Ram et al., 2024).

2.1. Radiometric Calibration

Radiometric calibration converts raw sensor intensity values into physically interpretable reflectance values. In laboratory settings and close-range hyperspectral imaging, this process is typically performed using dark and white reference measurements. The dark reference accounts for sensor-induced dark current, while the white reference represents the response obtained from a high-reflectance standard under identical illumination conditions. Subsequently, a relative reflectance image can be obtained by normalizing the raw image against these two reference values. This step is crucial because hyperspectral sensors record numerical intensity values influenced by illumination intensity and detector characteristics, rather than solely by reflectance properties (Lu et al., 2020).

Calibration quality depends on maintaining consistent data acquisition conditions. Variations in lamp intensity, sensor-to-target distance, imaging geometry, and surface orientation can lead to systematic differences between images. Therefore, calibration should ideally be performed under stable illumination and data acquisition geometry conditions, particularly when comparing spectra obtained from different samples or during different data acquisition sessions.

2.2. Noise Reduction and Spectral Preprocessing

Following radiometric correction, spectral preprocessing is typically applied to suppress noise, compensate for scattering effects, and enhance diagnostically significant spectral features. Smoothing methods, such as the Savitzky-Golay filter, are commonly used to reduce high-frequency noise while preserving the shape of absorption features. Standard Normal Variate (SNV) and Multiplicative Scatter Correction (MSC) are widely employed to mitigate additive and multiplicative effects arising from variations in surface structure, illumination, or sample geometry. First- and second-derivative transformations can further highlight subtle changes in spectral slope and absorption features; however, excessive differentiation may amplify noise if smoothing is inadequate (Rinnan et al., 2009).

No single preprocessing method is universally optimal for all agricultural hyperspectral datasets. The effectiveness of a method depends on the sensor, spectral range, target material, acquisition conditions, and analytical objective. Therefore, preprocessing should be considered an integral part of the analytical workflow rather than a fixed procedure applied uniformly to all datasets.

2.3. Background Removal and Region of Interest Selection

Segmentation and region of interest (ROI) selection are critical preprocessing steps because agricultural hyperspectral images often contain background materials in addition to the target plant tissue. Soil, trays, stems, shadows, and non-target objects can generate spectral signatures unrelated to the biological trait under investigation. Background removal can be performed using approaches such as thresholding, vegetation indices, clustering, principal component-based segmentation, or supervised classification (Ram et al., 2024).

ROI selection determines which pixels are retained for subsequent analysis. Depending on the objective, the analysis may be conducted using individual pixels, selected tissue regions, or average spectra derived from an entire ROI. Careful ROI definition enhances spectral purity and reduces unwanted variability; however, excessive averaging can suppress spatial heterogeneity that might otherwise contain diagnostically useful information. Therefore, the choice of segmentation and ROI strategies must align with the scale and purpose of the analysis.

3. FEATURE EXTRACTION AND DIMENSIONALITY REDUCTION

Hyperspectral images contain a large number of contiguous spectral bands, many of which are strongly correlated and may present redundant information. While this spectral richness is one of the key advantages of hyperspectral imaging, it increases computational complexity and can reduce model stability in situations where the number of variables exceeds the number of observations. Therefore, feature extraction and dimensionality reduction are crucial steps for transforming raw hyperspectral data into a more compact and information-rich representation (Bioucas-Dias et al., 2013; Li et al., 2019).

3.1. Spectral Feature Extraction

Spectral feature extraction aims to derive meaningful variables from reflectance spectra associated with the physiological, biochemical, or structural properties of plants. Common approaches include the use of raw reflectance values, first- and second-order derivatives, absorption features, spectral slopes, red-edge parameters and vegetation or narrow-band indices. These features can highlight subtle spectral differences that might not be easily detected in raw spectra alone; furthermore, they can improve the discrimination of plant conditions or the estimation of quantitative traits (Blackburn, 2007; Lu et al., 2020).

In agricultural applications, spectral indices are frequently constructed from selected combinations of wavelengths sensitive to pigments, water content, or structural characteristics. However, the effectiveness of an index depends heavily on the target variable, crop type, developmental stage, and data acquisition conditions. Therefore, the selection of spectral features should be based on both statistical suitability and physiological interpretability.

3.2. Band Selection and Dimensionality Reduction

Band selection reduces spectral dimensionality by retaining a subset of wavelengths that carry informational value while discarding redundant or noisy bands. While filter-based methods rank wavelengths according to statistical criteria, wrapper-based methods evaluate subsets of bands based on the performance of a predictive model. More advanced approaches may employ recursive feature elimination, optimization algorithms, or model-based

importance metrics. Due to the high correlation among hyperspectral bands, selected wavelengths tend to cluster in regions characterized by strong absorption features or high sensitivity to the target property (Ram et al., 2024).

Dimensionality reduction methods offer an alternative by transforming original spectral variables into a smaller number of derived components. Principal Component Analysis (PCA) is one of the most widely used approaches, as it captures a significant portion of spectral variance within a limited number of orthogonal components. Similarly, the Minimum Noise Fraction (MNF) transform is utilized to reorganize hyperspectral data based on signal-to-noise characteristics. Although these approaches can reduce computational load and suppress noise, the transformed components are often less directly interpretable than the original wavelengths (Bioucas-Dias et al., 2013; Vaddi et al., 2024).

3.3. Spectral–Spatial Features

Hyperspectral images provide not only spectral information but also spatial information regarding the arrangement and structure of neighboring pixels. Spatial features such as texture, shape, morphology, and local intensity variations can complement spectral features, particularly when symptoms or structural differences are spatially heterogeneous. While texture descriptors derived from gray-level co-occurrence matrices and morphological operations are among the traditional methods used to characterize spatial patterns, deep learning approaches can learn spectral-spatial features directly from image patches (Plaza et al., 2009; Li et al., 2019).

Integrating spectral and spatial information can enhance the discrimination between plant tissues or stress conditions by preserving both wavelength-dependent responses and local image structure. However, increased feature complexity also raises the risk of overfitting, especially in agricultural datasets with limited sample sizes. Therefore, the choice between purely spectral, spatial, and spectral-spatial representations should be guided by the biological objective, dataset size, and model complexity.

4. HYPERSPECTRAL IMAGE ANALYSIS AND MODELING

The analysis of hyperspectral data in precision agriculture typically involves the development of models that relate spectral or spectral-spatial information to plant characteristics, stress conditions, or categorical classes. Given that hyperspectral datasets are high-dimensional and often contain non-linear relationships, a wide range of approaches such as statistical methods, chemometrics, machine learning, and deep learning have been employed in this field. The choice of method depends on the prediction objective, sample size, dimensionality of the input data, and the need for model interpretability (Li et al., 2019; Lu et al., 2020).

4.1. Statistical and Chemometric Approaches

Traditional hyperspectral analyses often rely on multivariate statistical and chemometric methods. While Principal Component Analysis (PCA) is widely used for exploratory analysis and dimensionality reduction, Partial Least Squares Regression (PLSR) is extensively applied to predict continuous plant variables such as chlorophyll, nitrogen concentration, water-related properties, and biochemical constituents. For classification problems, Partial Least Squares Discriminant Analysis (PLS-DA) and Linear Discriminant Analysis (LDA) methods are employed. Although these approaches are computationally efficient and relatively interpretable, their performance may be limited when spectral relationships are strongly non-linear or when complex spatial patterns contribute significantly to class separation (Lu et al., 2020; Ram et al., 2024).

4.2. Machine Learning Methods

Machine learning methods offer greater flexibility in modeling non-linear relationships within hyperspectral datasets. Support Vector Machines (SVM), Random Forests (RF), k-Nearest Neighbors (KNN), and Artificial Neural Networks (ANN) are among the most frequently used algorithms in agricultural hyperspectral studies. SVM is particularly effective in high-dimensional spaces and can yield successful results with a relatively limited number of training samples; in contrast, RF offers robustness against noise and provides variable importance measures that can facilitate wavelength selection. However, the performance of these models remains heavily dependent on

preprocessing, feature selection, class balance, and the representativeness of the training dataset (Li et al., 2019; Lu et al., 2020).

4.3. Deep Learning Approaches

Deep learning is increasingly applied in hyperspectral image analysis due to its ability to learn hierarchical features directly from data and integrate spectral and spatial information. While one-dimensional convolutional neural networks (1D-CNNs) primarily model spectral patterns and 2D-CNNs focus on spatial structure, 3D-CNNs can simultaneously leverage both the spectral and spatial dimensions of the hyperspectral cube. More recently developed attention- and transformer-based architectures have further advanced the capability to model long-range spectral dependencies and complex image structures. However, deep learning typically requires larger training datasets, greater computational resources, and rigorous validation processes to mitigate overfitting and ensure model adaptability to new scenarios (Li et al., 2019; Ali et al., 2024).

In practical applications, there is no single modeling approach that is universally superior. Chemometric methods remain valuable when interpretability and limited sample sizes are critical; machine learning approaches offer a balance between flexibility and computational efficiency; and deep learning provides advantages, particularly when large datasets and spatial information are available. Therefore, model selection should be based not only on prediction accuracy but also on considerations regarding the biological objective, data structure, sample size, and intended operational use.

5. APPLICATIONS IN PRECISION AGRICULTURE

Hyperspectral imaging is utilized in a wide range of precision agriculture applications due to its ability to capture subtle spectral variations associated with plant physiology, biochemical composition, and structural characteristics. The primary application areas of this technology can be categorized into the assessment of plant health and stress status, the estimation of biochemical and biophysical properties, and analyses focused on phenotyping or quality. In these application areas, the fundamental analytical objective is generally to detect spectral or spectral-spatial patterns associated with plant responses of agricultural significance (Lu et al., 2020; Sarić et al., 2022).

5.1. Plant Health and Stress Assessment

One of the most widely researched applications of hyperspectral imaging is the detection of plant stress and diseases. Abiotic stresses such as drought, salinity, extreme temperatures, and nutrient deficiencies as well as biotic stresses caused by pathogens or pests, can alter pigment concentration, tissue structure, water status, and metabolic activity. These changes can affect reflectance patterns (spectral signatures) in the visible, red-edge, near-infrared, and shortwave infrared regions. Consequently, hyperspectral imaging can assist in distinguishing between healthy and affected tissues and, in some cases, detect spectral changes before severe visual symptoms appear (Lu et al., 2020; Sarić et al., 2022; García-Vera et al., 2024).

Disease detection studies typically employ a combination of spectral preprocessing, wavelength selection, and classification algorithms to differentiate infected tissues from healthy ones. Similarly, water stress assessment often relies on spectral regions sensitive to leaf water content and on reflectance variations linked to pigments and structure. Although high classification accuracy rates are frequently reported under controlled conditions, model performance may decline when lighting, cultivar, developmental stage, or symptom severity differ from those in the training dataset. This highlights the importance of robust validation methods and biologically interpretable spectral features in practical applications (Ram et al., 2024; García-Vera et al., 2024).

5.2. Biochemical and Biophysical Trait Estimation

Hyperspectral imaging is also widely used to estimate the biochemical and biophysical properties of plants through non-destructive methods. While chlorophyll and other pigments primarily influence reflectance in the visible and red-edge regions, water content and various biochemical constituents affect specific absorption features in the near-infrared and shortwave infrared regions. These relationships enable the estimation of variables such as chlorophyll concentration, nitrogen status, relative water content, leaf area-related traits, and other plant attributes using spectral indices, selected wavelengths, chemometric models, or machine learning techniques (Blackburn, 2007; Lu et al., 2020; Sarić et al., 2022).

Such estimations are particularly important for precision crop management, as they provide spatially resolved indicators of a plant's nutritional and physiological status without the need for destructive sampling. However, calibration relationships developed for a specific crop, cultivar, growth stage, or set of environmental conditions may not be directly transferable to others. Consequently, reliable estimations require the use of highly representative calibration datasets and the careful consideration of confounding factors such as canopy structure, illumination, and soil effects.

5.3. Phenotyping, Growth, and Quality Assessment

Hyperspectral imaging has become increasingly important in plant phenotyping because it enables the non-destructive and reproducible measurement of traits associated with growth, physiology, and stress responses. Spectral data can complement traditional morphological measurements by providing indicators related to pigment composition, tissue water status, and biochemical properties. This makes hyperspectral imaging particularly useful for screening genotypes, monitoring responses to treatments, and characterizing phenotypic variation, whether in controlled environments or field settings (Sarić et al., 2022).

Beyond phenotyping, hyperspectral imaging has also been employed to estimate traits related to biomass and growth and to assess agricultural produce quality. Spectral and spectral-spatial features can be correlated with plant vigor, maturity, internal composition, and both visible and invisible defects. Although the fundamental analytical principles remain consistent namely, deriving meaningful information by correlating spectral variations with reference measurements or categorical quality classes these applications extend the scope of hyperspectral imaging from crop monitoring to post-harvest assessment (Lu et al., 2020; Ram et al., 2024).

In the context of precision agriculture, the practical value of these applications depends on the ability to transform laboratory-scale spectral relationships into robust models that perform consistently under varying environmental and biological conditions. Therefore, future application-oriented studies should prioritize model adaptability to diverse conditions, the standardization of data acquisition and preprocessing procedures, and the integration of spectral information into agricultural decision-making processes.

6. CHALLENGES AND FUTURE PERSPECTIVES

Despite the significant potential of hyperspectral imaging in precision agriculture, various methodological and operational challenges continue to limit the widespread adoption of this technology. One of the most persistent challenges is the high dimensionality of hyperspectral data. While the presence of hundreds of contiguous bands increases storage and computational requirements, strong inter-band correlation can lead to redundant information and heighten the risk of overfitting. These issues become particularly critical in scenarios where the number of available samples is limited relative to the number of spectral variables (Bioucas-Dias et al., 2013; Li et al., 2019).

A second major challenge is the sensitivity of hyperspectral measurements to data acquisition conditions. Factors such as illumination variability, sensor calibration, imaging geometry, background effects, plant structure, and developmental stage can alter the recorded spectral response independently of the biological trait of interest. Consequently, models developed under controlled laboratory conditions may fail to maintain the same level of performance when adapted to different cultivars, environments, seasons, or data acquisition systems. This limitation underscores the need for standardized data acquisition protocols, representative calibration datasets, and independent validation studies conducted under diverse conditions (Lu et al., 2020; Sarić et al., 2022).

Future developments are expected to increasingly focus on robust spectral-spatial modeling, automated feature learning, and model transferability. While deep learning and attention-based architectures offer opportunities to leverage the full structure of hyperspectral data, transfer learning and self-supervised learning can reduce reliance on large-scale labeled datasets. At the same time, explainable AI is gaining increasing importance because predictive accuracy alone is insufficient when model decisions cannot be linked to physiologically meaningful wavelengths or plant traits (Li et al., 2019; Ali et al., 2024). Therefore, developing models that are both accurate and interpretable will be vital for fostering trust in hyperspectral decision-support systems.

Another significant trend is the establishment of more reproducible and standardized workflows. Even when analyzing similar datasets, variations in preprocessing, wavelength selection, segmentation, and validation strategies

can lead to markedly different results. Consequently, future studies should prioritize the transparent reporting of data acquisition conditions, preprocessing parameters, training-validation procedures, and model uncertainty. Such practices will enhance comparability across studies and facilitate the transition of hyperspectral imaging from experimental research to routine use in precision agriculture (Mishra et al., 2017; Ram et al., 2024).

CONCLUSIONS

Hyperspectral imaging has become a vital analytical tool in precision agriculture, as it enables the simultaneous acquisition of detailed spectral and spatial information from plant materials. This capability allows for the non-destructive characterization of subtle variations in pigment composition, water status, tissue structure, biochemical properties, and stress responses. However, the richness of information inherent in hyperspectral data also entails significant analytical complexity. Consequently, reliable interpretation depends on a well-designed processing workflow that encompasses appropriate calibration, spectral preprocessing, segmentation, feature extraction, dimensionality reduction, and model development.

The effectiveness of hyperspectral image analysis depends not only on the predictive performance of statistical, machine learning, or deep learning models but also on the quality and representativeness of the underlying data. Variations in illumination, sensor characteristics, plant species, cultivar, growth stage, and environmental conditions can significantly impact model robustness and transferability. Consequently, future progress will rely on more standardized acquisition and preprocessing procedures, transparent validation strategies, and models that combine prediction accuracy with physiological interpretability.

Overall, hyperspectral imaging offers significant potential for applications such as plant health assessment, stress detection, nutrient and pigment estimation, phenotyping, and quality evaluation. Continuous advancements in spectral-spatial analysis, automated feature extraction, and transferable modeling approaches are expected to enhance the practical value of this technology. However, wider adoption in precision agriculture will require the development of workflows that are reproducible, computationally

efficient, and agronomically meaningful, while capable of operating reliably under variable real-world conditions.

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CHAPTER 22

THE GUT–BRAIN AXIS IN CHICKENS: MECHANISMS, MICROBIOTA INTERACTIONS, AND IMPLICATIONS FOR HEALTH AND PRODUCTION

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INTRODUCTION

The gut–brain axis (GBA) is a bidirectional communication network linking the gastrointestinal tract and the central nervous system through neural, endocrine, immune, and metabolic pathways. Increasing evidence indicates that intestinal microbiota play a central role in this communication system by interacting with the enteric nervous system, immune cells, endocrine pathways, and the brain (Carabotti et al. 2015; Mayer et al. 2014). Consequently, alterations in gut microbial composition have been associated with changes in neurological development, stress responsiveness, behavior, immune homeostasis, and metabolic regulation across a wide range of animal species (Cryan and Dinan, 2012; Sharon et al. 2016; Morais et al. 2021).

In poultry, the gastrointestinal tract is a key determinant of nutrient utilization, feed efficiency, health, and welfare. The intestinal microbiota contributes to digestive function, pathogen exclusion, immune development, and maintenance of intestinal barrier integrity (Oakley et al. 2014; Kogut, 2019; Yadav and Jha, 2019). Although research on the microbiota–gut–brain axis has expanded considerably in mammals, knowledge of its role in avian species remains limited. Nevertheless, emerging evidence suggests that gut microbial communities can influence feeding behavior, stress responses, immune competence, and productive performance through interconnected neural, endocrine, and immune-mediated pathways (Lyte, 2014; Villageliu and Lyte, 2018; Kikusato and Toyomizu, 2023).

Stress is considered one of the major factors affecting gut–brain communication in poultry. Activation of the hypothalamic–pituitary–adrenal–interrenal (HPAI) axis can impair intestinal barrier integrity, alter microbial composition, and modulate immune function, ultimately affecting bird health, welfare, and productivity (Cao et al. 2021; Beldowska et al. 2023; Khongthong et al. 2023). Conversely, intestinal microorganisms produce bioactive metabolites, including short-chain fatty acids (SCFAs), neurotransmitter-like compounds, and amino acid derivatives, which may influence neuroendocrine signaling and behavioral responses (Beldowska et al., 2023). Recent poultry studies further indicate that microbiota modulation can affect stress-related behaviors, brain neurochemistry, intestinal function, and production traits (Huang et al. 2023; Johnson et al. 2024; Khongthong et al. 2023).

Growing restrictions on the use of antibiotic growth promoters have increased interest in nutritional strategies targeting the intestinal microbiota. Probiotics, prebiotics, synbiotics, phytogenic feed additives, and organic acids have been widely investigated because of their beneficial effects on microbial balance, intestinal integrity, immune function, and productive performance (Ricke et al. 2020; Shehata et al. 2022; Abdelli et al. 2021). However, their influence on microbiota–gut–brain communication and neuroendocrine regulation in poultry remains poorly understood (Beldowska et al. 2023).

Despite increasing evidence linking intestinal microbiota with host physiology and productivity, the mechanisms underlying the microbiota–gut–brain axis in poultry remain incompletely understood. Most studies have focused on intestinal health, microbial ecology, immunity, and growth performance, whereas the neurophysiological and behavioral consequences of microbiota modulation have received comparatively less attention (Beldowska et al. 2023; Kikusato and Toyomizu, 2023). Therefore, a comprehensive synthesis of current knowledge is needed to clarify the mechanisms of gut–brain communication in poultry and to identify key research priorities.

This review summarizes current knowledge of the microbiota–gut–brain axis in poultry, with particular emphasis on its structural components, communication pathways, roles in stress and welfare, implications for health and production, and future research perspectives

2. Components of the Avian Gut–Brain Axis

2.1. Neural Components: Central Nervous System, Enteric Nervous System and Vagus Nerve

The neural component of the avian gut–brain axis comprises the central nervous system (CNS), enteric nervous system (ENS), autonomic nervous system, and vagus nerve, which collectively mediate bidirectional communication between the gastrointestinal tract and the brain (Carabotti et al. 2015; Mayer et al. 2022). Signals originating from the intestinal environment are transmitted to the CNS, while brain-derived signals regulate gastrointestinal motility, secretion, immune activity, and microbial homeostasis.

The CNS integrates gut-derived information and coordinates physiological, behavioral, and neuroendocrine responses. In poultry, hypothalamic centers play a key role in regulating feed intake, energy balance,

and stress adaptation through activation of the hypothalamic–pituitary–adrenal–interrenal (HPAI) axis (Kikusato and Toyomizu, 2023). The ENS, often referred to as the "second brain," regulates intestinal motility, secretion, nutrient absorption, and mucosal function independently of direct central control (Furness, 2012).

The vagus nerve serves as the principal neural connection between the gut and brain. Through predominantly afferent fibers, it transmits information related to nutrient availability, microbial activity, and intestinal physiological status to the CNS (Carabotti et al. 2015; Forsythe et al. 2016). Although mechanistic evidence in poultry remains limited, available studies indicate that neural signaling contributes to digestive regulation, stress adaptation, and maintenance of physiological homeostasis (Beldowska et al. 2023; Kikusato and Toyomizu, 2023).

2.2. Intestinal Epithelium and Barrier Function

The intestinal epithelium forms the primary interface between the host and the luminal environment and serves as a critical component of the microbiota–gut–brain axis. This selectively permeable barrier facilitates nutrient absorption while preventing the translocation of pathogens, toxins, and harmful microbial products into systemic circulation (Turner, 2009; Mayer et al. 2022).

Barrier integrity is maintained through coordinated interactions among epithelial cells, mucus layers, tight junction proteins, and associated immune mechanisms. In poultry, preservation of epithelial integrity is essential for efficient nutrient utilization, disease resistance, and productive performance (Kogut, 2019; Yadav and Jha, 2019). Beyond its barrier function, the intestinal epithelium actively participates in microbial sensing and signaling through interactions with immune cells, enteroendocrine cells, and neural pathways.

Disruption of epithelial integrity can increase intestinal permeability, promote inflammation, and impair gut–brain communication. Consequently, maintenance of barrier function represents an important prerequisite for intestinal homeostasis and effective microbiota–host interactions (Chelakkot et al. 2018; Aburto and Cryan, 2024).

2.3. Immune System

The gastrointestinal tract contains the largest immune compartment in the body and plays a central role in microbiota–gut–brain communication. Gut-associated immune tissues continuously interact with intestinal microorganisms and contribute to immune development, pathogen defense, and maintenance of immune tolerance (Belkaid and Hand, 2014).

Microbial signals influence immune cell activity and cytokine production, while immune mediators can affect neuroendocrine pathways and brain function. Through these interactions, the immune system serves as an important intermediary between the intestinal microbiota and the central nervous system (Morais et al. 2021; Cryan et al. 2019). In poultry, maintenance of immune homeostasis is particularly important because immune activation is closely associated with growth performance, welfare, and disease resistance (Kogut, 2019).

Gut-associated lymphoid tissue (GALT) constitutes the major immune compartment of the avian gastrointestinal tract and plays a critical role in maintaining tolerance toward commensal microorganisms while mounting protective responses against pathogens. Cytokines produced by intestinal immune cells can influence neuroendocrine signaling and contribute to gut–brain communication.

2.4. Gut Microbiota

The gut microbiota constitutes a fundamental component of the avian gut–brain axis. It comprises diverse microbial communities that colonize the gastrointestinal tract and contribute to nutrient digestion, metabolic regulation, immune development, pathogen exclusion, and maintenance of intestinal barrier integrity (Oakley et al. 2014; Yadav and Jha, 2019).

The composition of the poultry gut microbiota is influenced by age, genetics, diet, environment, and management practices. Although microbial populations differ throughout the gastrointestinal tract, the ceca represent the primary site of microbial fermentation and metabolite production. Dominant bacterial groups typically include members of the Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria phyla (Oakley et al. 2014; Stanley et al. 2013).

Beyond their digestive functions, intestinal microorganisms produce bioactive metabolites and signaling molecules that influence neural, immune, and endocrine pathways. Consequently, the gut microbiota is increasingly recognized as an active regulatory organ that contributes substantially to gut–brain communication, health, welfare, and productive performance in poultry (Morais et al. 2021; Beldowska et al. 2023).

3. Communication Pathways of the Microbiota–Gut–Brain Axis

The microbiota–gut–brain axis functions through a complex network of neural, endocrine, immune, and metabolic signaling pathways that enable bidirectional communication between the gastrointestinal tract and the central nervous system. Through these interconnected mechanisms, intestinal microorganisms can influence host physiology, behavior, stress responsiveness, and metabolic regulation, while host-derived signals simultaneously shape intestinal function and microbial ecology (Carabotti et al. 2015; Morais et al. 2021; De Palma et al. 2014). Although many mechanisms have been characterized primarily in mammalian models, growing evidence suggests that similar pathways operate in poultry and contribute to health, welfare, and productive performance (Beldowska et al. 2023; Kikusato and Toyomizu, 2023).

3.1. Neural Pathways

Neural signaling represents one of the fastest communication routes within the microbiota–gut–brain axis. Information derived from the intestinal environment is transmitted to the central nervous system through the enteric nervous system, autonomic nervous system, and vagus nerve (Carabotti et al. 2015; Mayer et al. 2022). Gut microorganisms influence neural activity indirectly through microbial metabolites and signaling molecules that interact with enteroendocrine cells, immune cells, and enteric neurons. These interactions modulate vagal signaling and contribute to the regulation of appetite, gastrointestinal function, stress responsiveness, and behavior (Forsythe et al. 2016; Morais et al. 2021). In poultry, neural pathways are believed to play important roles in feed intake regulation, digestive physiology, and adaptation to environmental challenges, although mechanistic evidence remains limited (Kikusato and Toyomizu, 2023).

Recent evidence has revealed a more direct mechanism of gut–brain communication involving specialized enteroendocrine cells. These cells can function as sensory transducers by detecting luminal nutrients and microbial signals and rapidly transmitting information to the nervous system. In particular, Kaelberer et al. (2018) demonstrated that enteroendocrine cells form excitatory synapse-like connections with vagal afferent neurons, creating a direct neural circuit between the gut and the brain. This pathway enables rapid transmission of nutrient-derived signals through glutamatergic neurotransmission and provides an additional mechanism by which intestinal stimuli may influence feeding behavior, energy homeostasis, and neurophysiological responses.

3.2. Neuroendocrine Pathways (HPA/HPAI Axis)

Neuroendocrine communication is mediated primarily through the hypothalamic–pituitary–adrenal (HPA) axis and its avian counterpart, the hypothalamic–pituitary–adrenal–interrenal (HPAI) axis. Activation of this pathway results in corticosterone release, enabling physiological adaptation to stressors but potentially impairing intestinal integrity, immune function, and productive performance when activation is prolonged (Scanes, 2015; Kikusato and Toyomizu, 2023).

The intestinal microbiota can influence HPA/HPAI activity through microbial metabolites and signaling molecules, whereas stress-induced endocrine responses can alter intestinal permeability, microbial composition, and immune regulation (Cryan and Dinan, 2012; Morais et al. 2021). This reciprocal interaction highlights the close relationship between neuroendocrine signaling and microbial homeostasis within the gut–brain axis.

In addition to glucocorticoid signaling, enteroendocrine hormones including serotonin, glucagon-like peptide-1 (GLP-1), peptide YY (PYY), cholecystokinin (CCK), and ghrelin contribute to appetite regulation, gastrointestinal motility, energy metabolism, and stress adaptation. Together, these pathways form a major neuroendocrine link between the gut microbiota and host physiology (Mayer et al. 2022; Morais et al. 2021).

3.3. Immune-Mediated Communication

The immune system provides an important communication route linking intestinal microorganisms with the central nervous system. Microbial components and metabolites influence immune cell activity and cytokine production, while immune mediators can affect neuroendocrine pathways, neurotransmitter metabolism, and behavioral responses (Belkaid and Hand, 2014; Cryan et al. 2019).

Neural regulation of immunity also contributes to gut–brain communication. The concept of the inflammatory reflex proposes that neural circuits continuously monitor peripheral inflammation and regulate immune responses through autonomic pathways. In particular, the vagus nerve can modulate cytokine production through the cholinergic anti-inflammatory pathway, thereby limiting excessive inflammatory responses and maintaining physiological homeostasis (Tracey, 2009). This mechanism provides an important link between neural signaling and immune regulation within the microbiota–gut–brain axis.

Disruption of microbial homeostasis or intestinal barrier integrity may promote inflammatory signaling and immune activation, thereby affecting physiological and neurological functions. In poultry, immune-mediated communication is particularly relevant because environmental stressors, infectious challenges, and management conditions can simultaneously alter immune status and microbial composition (Kraimi et al. 2019; Cao et al. 2021).

3.4. Microbial Metabolite-Mediated Signaling

Microbial metabolites are key molecular mediators of microbiota–gut–brain communication. Among these, short-chain fatty acids (SCFAs), tryptophan-derived metabolites, bile acid derivatives, and neurotransmitter-like compounds have received particular attention because of their capacity to influence neural, endocrine, and immune pathways (Morais et al. 2021; Aburto and Cryan, 2024).

SCFAs contribute to intestinal barrier integrity, immune regulation, and metabolic homeostasis, whereas microbial modulation of tryptophan metabolism influences serotonin synthesis and neuroendocrine activity (Dalile et al. 2019; Yano et al. 2015). In addition, gut microorganisms can produce or modulate neuroactive compounds, including γ-aminobutyric acid (GABA),

catecholamines, and serotonin precursors, which indirectly affect host physiology through interactions with neural and immune pathways (Cryan et al. 2019).

Collectively, these metabolites provide a mechanistic link between intestinal microbial activity and systemic physiological regulation, making them central components of the avian microbiota–gut–brain axis.

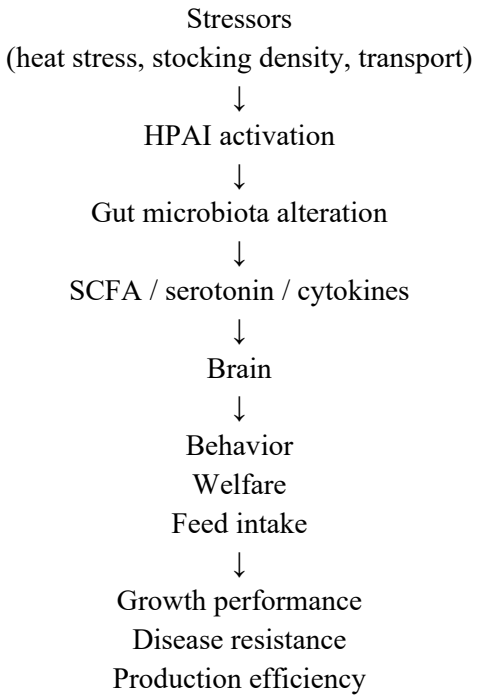

Figure 1. Simplified overview of microbiota–gut–brain communication and its consequences for poultry health and production.

4. Stress, Welfare and Behavioral Consequences

Stress is one of the most important factors influencing the microbiota–gut–brain axis in poultry. Environmental and management-related challenges can alter neuroendocrine activity, intestinal physiology, immune responses, and microbial homeostasis, thereby affecting welfare, health, and productive performance. Increasing evidence suggests that stress-induced disturbances of gut–brain communication contribute to many of the physiological and behavioral responses observed in commercial poultry systems (Kraimi et al. 2019; Beldowska et al. 2023).

4.1. Environmental and Management-Related Stressors

Commercial poultry production exposes birds to numerous environmental and management-related stressors, including high stocking density, transportation, feed restriction, handling procedures, social competition, poor air quality, and unfavorable housing conditions. These stressors activate neuroendocrine and immune pathways, alter intestinal function, and influence gut microbial composition (Kraimi et al. 2019; Kikusato and Toyomizu, 2023).

Stress-induced activation of the hypothalamic–pituitary–adrenal–interrenal (HPAI) axis may increase corticosterone secretion, alter intestinal permeability, and disrupt microbial homeostasis. Consequently, chronic exposure to environmental challenges can impair welfare, immune competence, and productive performance while promoting microbial dysbiosis and intestinal dysfunction (Cao et al. 2021; Beldowska et al. 2023).

4.2. Heat Stress and Gut–Brain Communication

Heat stress is among the most significant environmental challenges in poultry production. Elevated temperatures impair thermoregulation, reduce feed intake, compromise intestinal barrier integrity, and alter microbial composition (Lara and Rostagno, 2013; Cao et al. 2021). Reduced intestinal perfusion and oxidative stress can increase epithelial permeability, facilitating inflammatory responses and disturbances in gut–brain communication.

Heat stress-induced microbial dysbiosis may modify the production of microbial metabolites involved in immune and neuroendocrine regulation, thereby influencing stress responsiveness, welfare, and performance. Emerging evidence indicates that nutritional interventions capable of maintaining microbial homeostasis may improve resilience to thermal stress and mitigate its negative consequences (Abdelli et al. 2021; Kikusato and Toyomizu, 2023).

Overall, heat stress provides a clear example of how environmental challenges can disrupt multiple components of the microbiota–gut–brain axis simultaneously, linking intestinal dysfunction with impaired welfare and productive performance.

4.3. Behavioral Responses and Welfare Indicators

Behavioral responses represent important indicators of welfare and provide functional evidence of microbiota–gut–brain interactions in poultry. Alterations in gut microbial composition have been associated with changes in fearfulness, social interactions, exploratory behavior, and stress responsiveness (Cryan et al. 2019; Morais et al. 2021).

Among welfare-related behavioral disorders, feather pecking has received particular attention because of its association with microbial diversity, inflammatory signaling, and tryptophan metabolism (Meyer et al. 2013; Huang et al., 2023). Behavioral assessments such as tonic immobility, open-field, and novel object tests further suggest links between gut microbial communities and emotional reactivity in poultry (Van der Eijk et al. 2019; Fu et al. 2024). These findings support a role for the microbiota–gut–brain axis in behavioral regulation and welfare outcomes.

4.4. Microbiota Dysbiosis and Stress Adaptation

Microbial homeostasis is essential for maintaining effective gut–brain communication. Environmental and management-related stressors can disrupt microbial balance, leading to dysbiosis characterized by reduced microbial diversity and altered community structure (Hooks and O'Malley, 2017; Morais et al. 2021). Such alterations may impair microbial metabolite production, immune regulation, and neuroendocrine signaling, ultimately reducing stress resilience.

Conversely, a stable and diverse intestinal microbiota appears to enhance adaptation to environmental challenges by supporting barrier integrity, immune competence, and physiological homeostasis (Beldowska et al. 2023). Nutritional strategies designed to maintain microbial balance, including probiotics, prebiotics, synbiotics, and phytogenic feed additives, have shown potential to improve stress tolerance and welfare in poultry production systems (Abdelli et al. 2021; Kogut, 2019).

Overall, stress-induced modulation of the microbiota–gut–brain axis represents an important determinant of welfare, behavioral adaptation, and resilience in poultry.

5. Implications for Poultry Health and Production

The microbiota–gut–brain axis has important implications for poultry production because it influences physiological processes directly associated with health, welfare, resilience, and productive performance. Through coordinated interactions among intestinal microorganisms, neural pathways, immune responses, and endocrine signaling, gut–brain communication contributes to the regulation of growth, nutrient utilization, disease resistance, and adaptation to environmental challenges (Kogut, 2019; Beldowska et al. 2023).

5.1. Growth Performance and Feed Efficiency

Growth performance and feed efficiency are key determinants of profitability and sustainability in poultry production. Increasing evidence suggests that the microbiota–gut–brain axis contributes to productive performance through its effects on nutrient utilization, appetite regulation, metabolic homeostasis, stress adaptation, and intestinal health (Kogut, 2019; Yadav and Jha, 2019).

The intestinal microbiota enhances digestive efficiency through the production of bioactive metabolites that support intestinal development and metabolic regulation (Oakley et al. 2014; Stanley et al. 2013). Gut-derived signals may also influence feed intake and energy balance through interactions with neuroendocrine pathways and appetite-regulating hormones (Mayer et al. 2022; Morais et al. 2021). Consequently, alterations in microbial composition can affect nutrient utilization, feed conversion efficiency, and growth performance.

Conversely, stress-induced disturbances of gut–brain communication may impair productive performance by reducing feed intake, disrupting microbial homeostasis, and compromising intestinal function (Scanes, 2015; Cao et al. 2021). Current evidence therefore suggests that maintenance of a balanced microbiota may support optimal growth and feed efficiency in poultry production systems (Beldowska et al. 2023).

Table 1. Representative experimental studies investigating microbiota–gut–brain axis interactions in poultry.

Study	Species	Intervention	Main Findings
Kraimi et al., 2019	Japanese quail	Cecal microbiota transfer	Microbiota transfer altered emotional reactivity, providing evidence for a causal relationship between gut microbiota and behavioral responses.
Huang et al., 2023	Laying hens	Assessment of feather pecking, gut microbiota, and serotonin metabolism	Feather pecking behavior was associated with microbial dysbiosis, impaired serotonin metabolism, and altered inflammatory responses.
Johnson et al., 2024	Laying hens	Dietary synbiotic supplementation	Synbiotic supplementation modulated gut microbiota, reduced stress-related behaviors, and altered brain monoamine concentrations.
Khongthong et al., 2023	Broiler chickens	Sanguinarine-based isoquinoline alkaloids under heat stress	Improved growth performance, intestinal integrity, and gut–brain axis regulation during heat stress.
Fu et al., 2023	Heat-stressed chickens	Bacillus subtilis supplementation	Alleviated hippocampal neuroinflammation and improved gut microbial homeostasis under heat stress.
Fu et al., 2024	Egg-laying chickens	Early-life cecal microbiota transplantation	Reduced social stress and injurious behaviors, suggesting long-term programming effects of early microbiota colonization.

Hieke et al., 2019	Chickens	Extended photoperiod regimen	Circadian disruption altered microbiota acquisition and community composition during early development.
Song et al., 2014	Broiler chickens	Probiotic supplementation during heat stress	Enhanced intestinal morphology, microbial balance, and epithelial barrier integrity.
Meyer et al., 2013	Laying hens	Comparison of feather-pecking phenotypes	Behavioral phenotypes were associated with differences in intestinal microbial metabolites.
Van der Eijk et al., 2019	Laying hens	Divergent selection for feather pecking behavior	Feather-pecking lines exhibited distinct gut microbiota profiles associated with behavioral traits.

5.2. Intestinal Health and Disease Resistance

Intestinal integrity and immune competence are essential for maintaining health and productivity in poultry. The microbiota–gut–brain axis contributes to these processes through interactions among intestinal microorganisms, epithelial barriers, immune cells, and neuroendocrine pathways (Belkaid and Hand, 2014; Kogut, 2019).

A balanced intestinal microbiota supports barrier function, regulates inflammatory responses, and enhances resistance to pathogenic microorganisms. Conversely, microbial dysbiosis may promote inflammation, impair nutrient absorption, and increase susceptibility to enteric disorders and systemic infections (Turner, 2009; Aburto and Cryan, 2024). In addition, stress-induced activation of neuroendocrine pathways can further compromise immune competence by altering microbial composition and intestinal integrity (Cao et al. 2021).

This is particularly important in poultry production, where enteric pathogens such as Salmonella spp., Escherichia coli, Clostridium perfringens, and Campylobacter spp. remain major health and economic concerns. A balanced intestinal microbiota may limit pathogen colonization through competitive exclusion, production of antimicrobial compounds, and stimulation of protective immune responses (Kogut, 2019; Ricke et al. 2020).

The intestinal microbiota also contributes to disease resistance through competitive exclusion of pathogens and stimulation of protective immune responses. Therefore, maintenance of microbial homeostasis represents an important component of sustainable disease prevention and flock health management (Ricke et al. 2020; Yadav and Jha, 2019).

5.3. Early-Life Microbiota Programming and Development of the Gut–Brain Axis

The establishment of the intestinal microbiota begins immediately after hatch and plays a critical role in the development of the gastrointestinal tract, immune system, and gut–brain communication pathways. Early microbial colonization influences intestinal maturation, immune development, and physiological adaptation, suggesting that the post-hatch period represents a critical window for long-term programming of host responses (Oakley et al. 2014; Stanley et al., 2013).

Evidence from animal studies indicates that early-life microbial communities may influence neurodevelopment, stress responsiveness, and behavioral regulation through programming effects on the gut–brain axis (Cryan et al. 2019; Morais et al., 2021). Although poultry-specific evidence remains limited, interventions such as probiotics, synbiotics, competitive exclusion cultures, and in ovo applications have demonstrated potential to promote beneficial microbial establishment and improve health, resilience, and productive performance (Abdelli et al. 2021; Kikusato and Toyomizu, 2023).

These findings suggest that early microbial programming may represent a valuable strategy for improving long-term poultry health and productivity.

5.4. Circadian Rhythm and the Microbiota–Gut–Brain Axis

Circadian rhythms regulate numerous physiological processes, including metabolism, immune function, feeding behavior, and hormone secretion. Emerging evidence indicates that bidirectional interactions between host circadian systems and intestinal microbial communities represent an important component of the microbiota–gut–brain axis (Thaiss et al. 2016; Voigt et al. 2016).

In poultry, photoperiod is a major environmental factor influencing growth, reproduction, welfare, and behavior through regulation of melatonin secretion and neuroendocrine activity (Cassone, 2014; Scanes, 2015). Recent studies have shown that microbial composition and metabolite production exhibit daily oscillations influenced by feeding patterns and host physiology (Liang et al. 2015; Thaiss et al. 2016). Disruption of circadian rhythms may therefore alter microbial homeostasis, intestinal function, and gut–brain communication. In poultry, experimental evidence has demonstrated that extended photoperiod regimens can modify microbiota acquisition and community structure during early development, indicating that lighting programs may directly influence host–microbiota interactions and physiological adaptation (Hieke et al. 2019).

Although evidence in poultry remains limited, understanding the interactions among photoperiod, microbial rhythms, and physiological regulation may provide novel opportunities to optimize lighting programs, feeding schedules, and management strategies aimed at improving health, welfare, and production efficiency (Kikusato and Toyomizu, 2023).

6. Knowledge Gaps and Future Perspectives

Despite growing interest in the microbiota–gut–brain axis in poultry, current knowledge remains considerably less advanced than in mammalian species. Most poultry studies have focused on intestinal health, microbial composition, nutrient utilization, and productive performance, whereas the neurophysiological and behavioral mechanisms underlying gut–brain communication remain poorly understood (Beldowska et al. 2023). Consequently, many current concepts are extrapolated from mammalian models, highlighting the need for poultry-specific mechanistic studies.

A major research priority is the identification of causal relationships between specific microbial taxa, microbial metabolites, and host physiological responses. Although associations have been reported between gut microbiota composition, serotonin metabolism, inflammatory signaling, stress responses, and behavioral traits such as feather pecking, direct evidence of causality remains limited (Huang et al. 2023; Fu et al. 2024). Future studies should integrate microbiota manipulation approaches, microbial transplantation, and targeted metabolomics to clarify these relationships.

Another important knowledge gap concerns early-life programming of the microbiota–gut–brain axis. The post-hatch period is a critical developmental window during which microbial colonization, immune maturation, and neuroendocrine development occur simultaneously. Understanding how hatchery conditions, in ovo interventions, and early nutritional strategies influence long-term health, welfare, and resilience may provide valuable opportunities for improving poultry production (Stanley et al. 2013; Kogut, 2019; Kikusato and Toyomizu, 2023).

Further research is also needed to clarify the roles of microbial metabolites, circadian regulation, and host–microbiota interactions in gut–brain communication. Integrative multi-omics approaches combining metagenomics, metatranscriptomics, metabolomics, and systems biology are likely to provide deeper insights into the molecular mechanisms underlying microbiota–gut–brain signaling in poultry (Morais et al. 2021; Cryan et al., 2019; Aburto and Cryan, 2024).

From an applied perspective, microbiota-targeted nutritional interventions and the identification of microbial biomarkers associated with stress resilience, welfare, disease resistance, and productive performance

represent promising areas for future research. Such advances may facilitate the development of precision nutrition and microbiota-based management strategies aimed at improving poultry health, welfare, and sustainable production efficiency (Abdelli et al. 2021; Johnson et al., 2024; Beldowska et al. 2023).

Emerging technologies may provide new opportunities for advancing microbiota–gut–brain research in poultry. Precision livestock farming tools, artificial intelligence-based behavioral monitoring systems, and microbiome-informed nutritional strategies could facilitate the identification of microbial biomarkers associated with stress resilience, welfare, and productive performance. Integration of these technologies with microbiome and metabolome datasets may contribute to the development of precision poultry production systems in the future.

7. Conclusions

The microbiota–gut–brain axis is an important regulatory network linking the gastrointestinal tract and the central nervous system through neural, endocrine, immune, and metabolic pathways. Although research in poultry remains less advanced than in mammals, accumulating evidence indicates that intestinal microbial communities play a central role in regulating gut health, immune competence, stress responsiveness, behavior, and productive performance (Cryan and Dinan, 2012; Cryan et al. 2019; Morais et al. 2021).

Current findings suggest that gut–brain communication is influenced by complex interactions among the intestinal microbiota, intestinal barrier, neuroendocrine pathways, immune mediators, and microbial metabolites. Disturbances in microbial homeostasis, particularly under environmental and management-related stressors, may impair intestinal integrity, immune regulation, welfare, and production efficiency (Cao et al. 2021; Beldowska et al. 2023). Conversely, maintaining a balanced microbiota appears to support physiological resilience and overall flock performance.

The microbiota–gut–brain axis also represents a promising target for nutritional and management interventions. Strategies designed to modulate intestinal microbial communities, including probiotics, prebiotics, synbiotics, phytogenic feed additives, and other functional dietary approaches, may

improve health, welfare, and productivity by supporting gut–brain communication pathways (Abdelli et al. 2021; Johnson et al. 2024).

Despite recent advances, major knowledge gaps remain regarding the causal mechanisms linking microbial communities with neuroendocrine, immune, and behavioral responses in poultry. Future studies integrating microbiomics, metabolomics, neurophysiology, and systems biology approaches will be essential for clarifying these relationships and translating scientific knowledge into practical applications.

Overall, a better understanding of microbiota–gut–brain interactions may provide new opportunities to improve poultry health, welfare, resilience, and sustainable production efficiency, making the gut–brain axis an increasingly important area of poultry research.

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CHAPTER 23

STRUCTURAL ANALYSIS OF THE LIQUID ORGANIC AND MINERAL FERTILIZER MARKET IN THE TR71 REGION-TÜRKİYE: COMPETENCES, MARKET DYNAMICS, AND PRODUCER TRENDS FROM A DEALER PERSPECTIVE[1]

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[1] This study was compiled from the data of the master's thesis titled "***Evaluation of the Liquid Organic and Mineral Fertilizer Market in the TR71 Region***" completed by Gökhan EVNİ in 2025 at Kırşehir Ahi Evran University, Institute of Science, Department of Agricultural Economics.

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INTRODUCTION

The increasing global population and the adverse conditions brought about by climate change highlight the importance of area-based productivity in agricultural production. Although the intensive use of chemical fertilizers in conventional production models, implemented since the second half of the 20th century, provided short-term yield increases, it has eventually caused deterioration in the physical, chemical, and biological structure of the soil (Nandwa & Bekunda, 1998; Diacono & Momtemurro, 2010; Kılıç & Korkmaz, 2012; Bhat et al., 2019; Meena et al., 2019). The long-term and unbalanced use of solid chemical fertilizers has led to environmental problems such as soil salinization (Zhang et al., 2015), pH changes (especially acidification) (Ojeniyi, 2002; Zhang et al., 2015; Rusu et al., 2023), and nitrate accumulation in groundwater resources (Kızılaslan & Kızılaslan, 2005; Kılıç & Korkmaz, 2012; Jalali & Jalali, 2020). This situation has brought the concept of "soil health" to the forefront, which considers the soil as a living ecosystem alongside agricultural yield (Doran & Zeiss, 2000; Lehmann et al., 2020). Soil health is defined as the capacity of the soil to sustainably support plant and animal production, and to regulate water and nutrient cycles (Doran & Zeiss, 2000; Lehmann et al., 2020). In this context, liquid organic, organomineral, and mineral fertilizers, which can be applied through drip irrigation systems and have high plant absorption rates, are used as alternative inputs to preserve soil structure and enhance nutrient use efficiency (Martínez-Alcántara et al., 2016; Li et al., 2021; Shi et al., 2024; Yıldız & Dizikısa, 2026).

Concerns regarding soil health are addressed with concrete targets in international agricultural policies. The Farm to Fork Strategy, published under the European Green Deal, aims to reduce nutrient losses in agricultural areas by at least 50% and decrease total fertilizer use by at least 20% by 2030 (European Commission, 2020). The 2030 EU Soil Strategy document, prepared to support these goals, commits to restoring all soils within EU borders to a healthy condition by 2050 (European Commission, 2021). The Soil Monitoring Law proposal (COM(2023) 416), presented in 2023, aims to establish a legal framework for the regular monitoring and improvement of soil health in member states (European Commission, 2023). These regulations necessitate preventing the loss of soil organic matter and expanding the use of inputs that

have low environmental emissions and are easily absorbed by plants (European Commission, 2021; European Commission, 2023).

The fact that the organic matter ratio is below 2% in a vast majority of cultivated agricultural lands in TÜRKİYE (Sönmez et al., 2018) limits the availability of applied solid chemical fertilizers (Adekiya et al., 2019). To improve this situation, the Ministry of Agriculture and Forestry created support items for the use of organic and organomineral fertilizers for the first time in 2019 (with the first payment in 2020) and implemented the Fertilizer Tracking System (GTS) with a communiqué published in the Official Gazette on April 6, 2017, to combat counterfeit products and track inputs (Official Gazette, 2017). Additionally, the expansion of drip irrigation infrastructure in water-scarce regions has directly influenced the use of liquid fertilizers applied through irrigation water (fertigation).

The retail sale and delivery of agricultural inputs to producers are carried out through fertilizer dealers. A significant portion of producers in Türkiye purchase fertilizers without conducting soil analysis and rely on dealer recommendations for application rates (Kızılaslan & Kızılaslan, 2005). This makes the dealers' technical knowledge, professional experience, and product preferences a determining factor in the field adoption of new fertilizer technologies.

The TR71 Region, which includes the provinces of Aksaray, Kırıkkale, Kırşehir, Nevşehir, and Niğde according to the Nomenclature of Territorial Units for Statistics, is one of the areas with intensive production of cereals, potatoes, and sugar beet (TÜİK, 2026). The agricultural production structure, large enterprise scales, and the use of drip irrigation systems in the region have made the liquid fertilizer market a subject of investigation. Furthermore, studies conducted in the region provide evidence that such innovative products can be effective in increasing soil organic matter and offer significant savings in various areas. Conversely, due to inadequate product inspections, items with incorrect or inappropriate contents can enter the market, creating unfair competition; however, these products create new employment opportunities and positively affect the market volume of complementary goods (Evni & Kan, 2024).

Research on fertilizers in the literature has primarily focused on producer-level demand, while studies examining the role and sales behaviors

of retail dealers providing input distribution in the liquid fertilizer market remain limited. In this study, the socio-economic characteristics of 89 fertilizer dealers in the TR71 Region (Aksaray, Kırıkkale, Kırşehir, Nevşehir, and Niğde provinces), the share of liquid organic and mineral fertilizers in the dealers' total turnover, and the factors affecting their sales behaviors were examined using quantitative methods. The obtained data aims to reveal the structure of the regional liquid organic and mineral fertilizer input market and provide actionable assessments for the sector.

MATERIAL AND METHODS

The primary data source of the research consists of data collected through surveys from dealers trading liquid organic and mineral fertilizers in the provinces of Aksaray, Kırıkkale, Kırşehir, Nevşehir, and Niğde within the TR71 Region. Previously published scientific articles, books, academic reports, and current statistical records provided by official institutions constitute the secondary data set of the research.

It was determined that there are 262 commercial enterprises selling liquid organic and mineral fertilizers across the region. While determining the sample size, the objective was to reach a volume covering at least one-third of the population. Accordingly, purposive sampling, a non-random sampling approach, was preferred. Purposive sampling refers to the selection of units capable of representing the population based on a specific purpose and judgment (Güneş & Arıkan, 1988). In this method, the researcher acts as the decision-maker on which units will be included in the sample, relying on their theoretical knowledge and field experience (Çiçek & Erkan, 1996). Criteria such as voluntariness, accessibility, and the ability to represent the research area were considered during the enterprise selection phase. Based on the opinions of experts working in provincial and district Directorates of Agriculture and Forestry, face-to-face surveys were conducted with a total of 89 enterprise representatives across five provinces. This study was compiled from the data of the master's thesis titled "Evaluation of the Liquid Organic and Mineral Fertilizer Market in the TR 71 Region," completed by Gökhan EVNİ in 2025 at Kırşehir Ahi Evran University, Institute of Science, Department of Agricultural Economics (Evni, 2025). The research area (Figure 1) and the

number of samples selected according to the provinces in the research area are presented in Table 1.

Table 1: Distribution of Sample Size by Province

Province Name	Total Number of Commercial Enterprises Selling Liquid Organic and Mineral Fertilizers	Percentage Distribution (%)	Number of Commercial Enterprises Included in the Sample
Aksaray	61	23,28	20
Kırıkkale	20	7,63	7
Kırşehir	41	15,65	14
Nevşehir	59	22,52	21
Niğde	81	30,92	27
Total Enterprises	**262**	**100.00**	**89**

Source: Kırşehir Provincial Directorate of Agriculture and Forestry Records, 2023

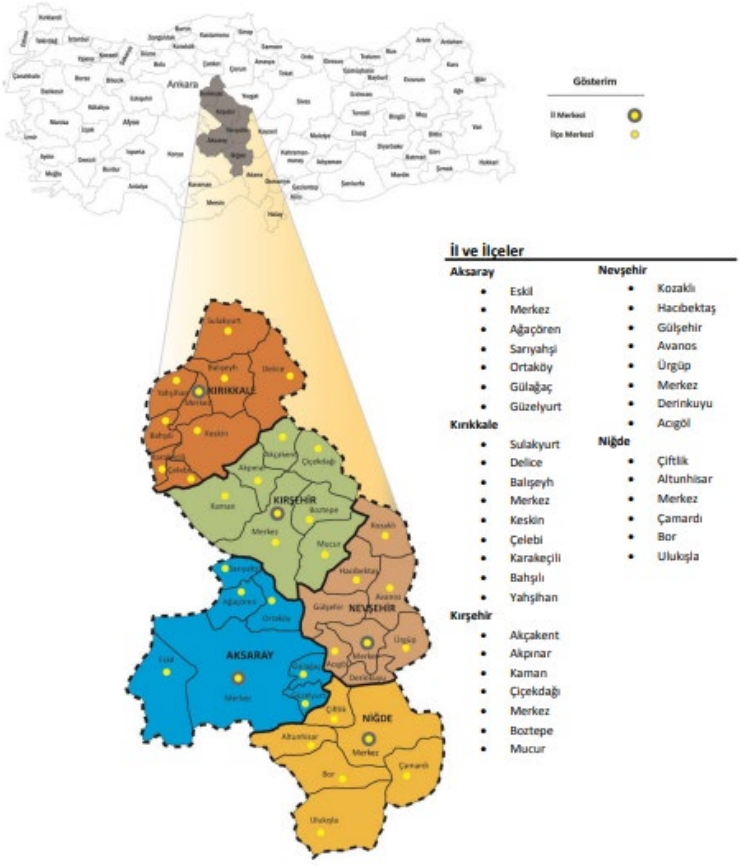

Figure 1: Map of provinces and districts in the TR71 Region-Türkiye

The research design is based on quantitative data collection and analysis techniques. A structured survey questionnaire was used as the data collection tool. The questionnaire consists of variables such as the socio-demographic characteristics of the business owners, the fields of activity and commercial experiences of the enterprises, as well as questions measuring the operators' perceptions regarding corporate audit processes, product profitability, and producer behaviors. Previous similar studies were utilized in designing the questionnaire, and content validity was ensured by evaluating the questions with expert opinions (Bryman, 2016).

The data obtained from the field were analyzed using a statistical software package. In the initial examination of the data set, descriptive statistics were obtained by calculating frequencies, percentage distributions, and arithmetic means. Appropriate models for the data type were selected to test whether the examined variables showed a statistically significant difference between provinces. The Chi-Square ($\chi 2$) test was used for independence checks between discrete categorical variables (Kesici & Kocabaş, 1998). One-Way Analysis of Variance (ANOVA - F test) was preferred to determine the statistical significance of differences between the means of continuous variables, and the Duncan Multiple Range Test was applied to identify homogeneous subgroups when the difference was significant. For the province-based comparisons of ordinal perceptual data that did not fully meet parametric test assumptions, the Kruskal-Wallis H test was utilized.

RESULTS AND DISCUSSION

When the demographic characteristics and educational profiles of operators active in the liquid organic and mineral fertilizer market of the TR71 Region are evaluated together, it is evident that the sector is transforming into a professionalized structure centered on technical expertise. The general average age of the operators being around 40 and the vast majority of participants being university graduates prove that the market is driven by a dynamic group of actors capable of following innovations. Statistical analyses comparing age and educational status on a provincial basis indicate a significant regional divergence for both variables at a 90% confidence interval. This divergence is important as it reflects the transition between the traditional commercial approach and the new generation technical sales model in the

sector. For instance, operators in Kırıkkale province represent a more traditional structure with an average age of approximately 49 and a predominance of high school-level education. In contrast, the average age dropping below 40 and the significantly high number of university graduates in provinces like Nevşehir and Aksaray indicate that a young and highly educated new generation is beginning to dominate the sector (Table 2).

Table 2. Age and Educational Status of Operators by Province

	Provinces					**Total**
	Nevşehir	**Niğde**	**Aksaray**	**Kırıkkale**	**Kırşehir**	
Operator's Age (Years)	38.43	41.22	39.30	49.14	40.14	40.58
	F:2.07		**p:0.09**	**H_0:Reject**		
Educational Status (Persons)	**Provinces**					**Total**
	Nevşehir	**Niğde**	**Aksaray**	**Kırıkkale**	**Kırşehir**	
High School	5	11	4	6	7	33
Associate Degree	0	1	1	0	0	2
Bachelor's Degree	16	15	15	1	7	54
Total	**21**	**27**	**20**	**7**	**14**	**89**
	χ^2:14.01		**p:0.08**	**H_0:Reject**		
Departments (Person)						
Plant Protection	10	8	6	0	2	26
Field Crops	5	1	4	1	2	13
Horticulture	1	1	3	0	1	6
Business Administration	0	3	1	0	1	5
Biology	0	1	0	0	0	1
Faculty of Education	0	1	0	0	0	1
Public Administration	0	1	0	0	0	1
Animal Science	0	0	0	0	1	1
Total	**16**	**16**	**14**	**1**	**7**	**54**

The greatest contribution of this regional dynamic between education level and age to the market structure emerges in the areas of expertise of university graduate operators. The fact that 45 of the 54 operators who

completed their undergraduate education graduated directly from agricultural engineering disciplines such as plant protection, field crops, and horticulture directly affects the quality of the market (Table 2). The low number of graduates from non-agricultural fields indicates that technical knowledge has become a mandatory entry barrier to operate in this market. These professionals with an agricultural background act as technical guides providing field consultancy to producers, rather than merely acting as commercial sellers. Therefore, when education level and agricultural expertise are evaluated together with the age factor, it is seen that the fertilizer market in the TR71 Region is ceasing to be merely a commercial trading area and points to the presence of a technical team with the potential to bring producers together with accurate information.

When the sectoral orientations of the enterprises operating in the TR71 Region and their years of experience in these fields are evaluated together, it is understood that the market has a deep-rooted structure specialized in specific areas. Examining the primary fields of activity of the enterprises, it is observed that sales are concentrated primarily in Plant Protection Products (PPP) and secondarily in fertilizers (Figure 2). This sectoral focus in the market is supported by a solid average business experience of 13.28 years across the region (Figure 3).

When comparing the experience durations of operators in their active fields of activity by province, the calculated F value (0.49) shows no statistically significant difference among the provinces. This confirms that enterprises in each province share a similar background and an established commercial culture in the market. This long-term field experience, especially combined with university-level agricultural engineering education, proves that operators are not merely commercial intermediaries in the sector. On the contrary, it becomes clear that these actors are experienced field consultants who are closely acquainted with the region's agricultural dynamics and producers' problems, capable of offering reliable technical solutions during the production process.

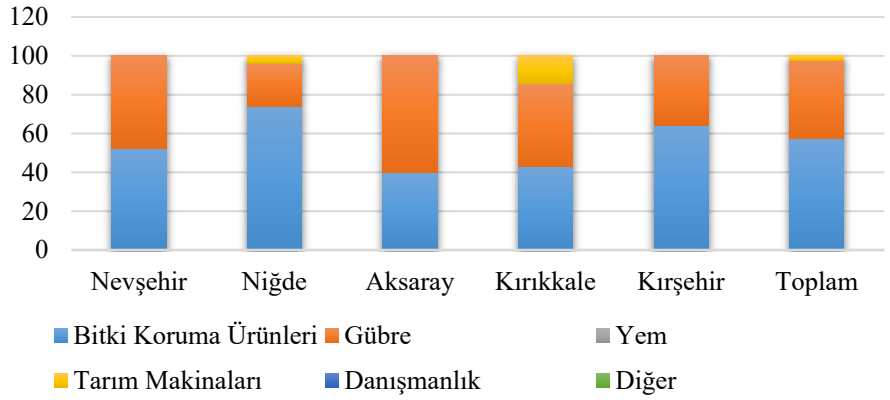

Figure 2. Fields of Activity of Operators by Province (%)

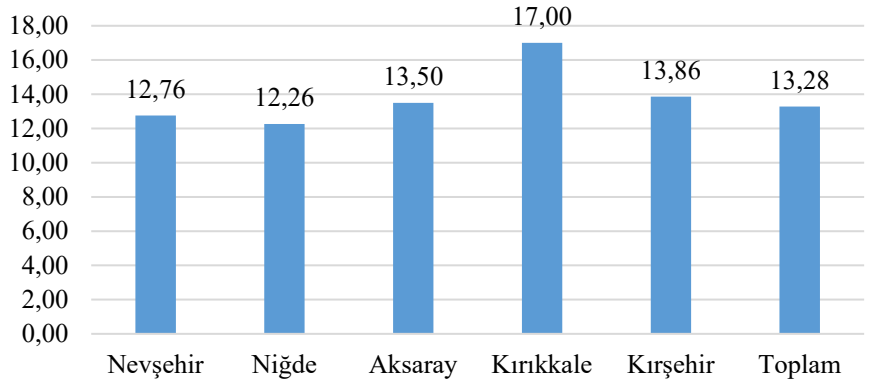

Figure 3. Business Experience of Operators Selling Liquid Organic and Mineral Fertilizers in Active Fields of Activity by Province (Years)

When examining the levels of importance attributed to the sale of liquid organic and mineral fertilizers by commercial enterprises operating in the TR71 Region, it is understood that this product group holds a very high commercial value in the sector. According to the data in Figure 4, 88.76% of the enterprises across the region defined this activity as "very important," while 7.87% defined it as "important". The proportion of those who found the activity "unimportant" remained at a mere 1.12%, while no operator selected the "not important at all" option. This emerging picture confirms that the liquid fertilizer trade is a strategic activity area and a primary income source for the companies.

Comparisons made on a provincial basis provide important findings regarding regional dynamics. All enterprises in Aksaray, Kırıkkale, and

Kırşehir provinces characterized the activity in question as "very important" at a rate of 100%. The situation is quite similar in Nevşehir province, where 95.24% of the enterprises evaluated the activity as "very important". The strong consensus observed in these four provinces shows that the enterprises are highly committed to the liquid fertilizer market. Niğde province, on the other hand, stands as the only example slightly diverging from this general trend. While 66.67% of the enterprises in Niğde found this area "very important," there were participants who remained undecided or expressed negative opinions at lower rates. As a result of the Chi-Square analysis applied to test the relationship between the variables, the p-value was calculated as 0.08. This statistical result proves a significant difference in the levels of attributed importance among the provinces at the 90% confidence interval. This divergence, which leads to the rejection of the H0 hypothesis and is largely driven by Niğde province, reveals that regional needs and local market strategies are not homogeneous in structure.

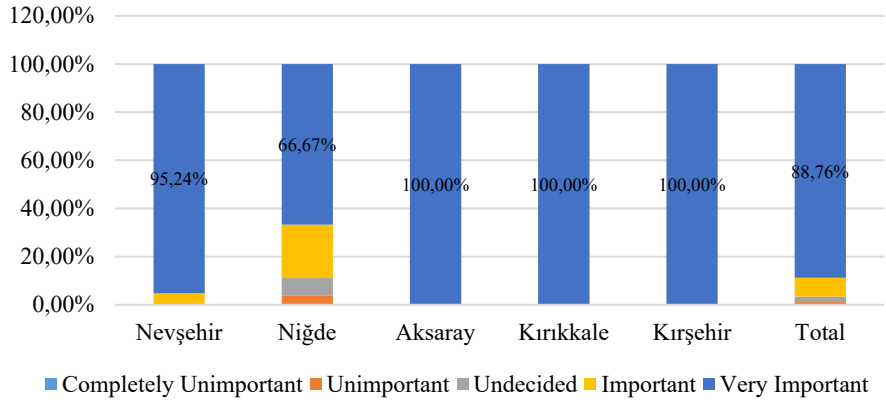

Figure 4. Importance of Liquid Organic and Mineral Fertilizer Activity for Enterprises by Province (%)

When the dynamics of the liquid organic and mineral fertilizer market in the TR71 Region are examined through the perceptual and cognitive evaluations of the operators, a statistically significant asymmetry is found between the dealers' levels of professional self-efficacy and their trust in the institutional functioning of the market. Examining the attitudes toward market entry barriers and the adequacy of authorization processes, it was determined that operators do not view the existing criteria as an institutional assurance

mechanism. Indeed, the perception of deficiency in dealership authorization processes exhibits a statistical regional difference at the 99% confidence level (χ^2=33.78), with 60% of participants in Aksaray and 71.43% in Kırıkkale stating that current regulations are inadequate. Similarly, doubts about the effectiveness of market surveillance and inspection mechanisms (χ^2=20.58), along with criticisms regarding the weakness of statistical recording systems (χ^2=23.14), point to structural problems in the sector.

Despite this low trust profile towards the institutional structure, complete homogeneity and high self-confidence are observed in the dealers' individual professional competences and technical consulting capacities. This situation is explained by the "information asymmetry" theory frequently encountered in agricultural input markets. Agricultural inputs fall into the category of "experience goods," the quality of which can only be understood after use. As emphasized in the study by Bold et al. (2017), inspection weaknesses and quality uncertainties in input markets cause dealers to bypass institutional mechanisms in the field and establish themselves as the primary technical authority.

When the financial dynamics and value chain of the sector are analyzed, it is found that the perception of commercial return scales according to regional competition conditions. The Kruskal-Wallis test results prove that the perception of high liquid fertilizer profit margins contains a statistical difference on a provincial basis (p=0.03). Dealers especially in Kırıkkale, Aksaray, and Niğde express the relative profitability of liquid organic and mineral fertilizers more dominantly. This economic rationality coincides with the micro-level reflections of macroeconomic regulations on agricultural inputs. For example, in an econometric study on the fertilizer market in Türkiye by Çobanoğlu et al. (2021), it was determined that the VAT exemption applied to inputs created an effect that expanded the profit margins of actors in the supply chain, rather than directly reducing producer costs. The positioning of liquid fertilizers in an innovative segment lowers price elasticity, allowing sellers to implement "value-based pricing" strategies and protect their profitability margins.

When the input purchasing and usage behaviors of the producer from the dealer perspective are examined, regional differences consistent with the Technology Acceptance Models (TAM) are identified. The variance analysis

(F=6.38) conducted on the level of conscious use reveals a significant regional variance at the 99% confidence interval. According to the operators' assessments, the tendency to use liquid fertilizer is associated with the scale of the cultivated land rather than the direct wealth level of the producer. This rational investment tendency aligns perfectly with the findings of McCormack et al. (2021) on the adoption of agricultural nutrition innovations. The relevant literature confirms that enterprises with economies of scale integrate liquid inputs that provide ease of application into their production processes more rapidly, anticipating perceived usefulness.

However, there are notable cognitive dissonances in producer behaviors. The tendency of producers to accurately analyze their agricultural needs in fertilizer selection (F=3.13) and their behavior of examining the label and content information of the purchased product (F=3.67) show inter-provincial divergence at the 95% confidence level. While producers in Kırşehir province receive a high average score of 3.71 in label reading, the perception of these same producers acting with knowledge of specific plant needs remains at the 2.43 level, indicating that there are serious gaps in translating visual access to information (label inspection) into agronomic practice (needs analysis).

When the factors shaping purchasing decisions in the agricultural input market are analyzed, it is determined that trust- and reference-based relationship marketing takes precedence over direct price sensitivity. Among the factors determining producer preferences, it was found that the strongest decision determinants are the product being branded and reliable (4.49) and dealer guidance (4.45). Purely economic triggers such as the relative inexpensiveness of liquid fertilizers (4.26) and cost increases in other fertilizer types (4.20) lag behind these trust elements. This situation is explained by the concepts of relational benefits and "face" (reputation) in rural sociology. As confirmed by the research conducted by Gao et al. (2023), in agricultural input purchases, producers make repurchase intention decisions leaning on face-to-face trust relationships, social capital, and the relational benefits offered by the dealer, rather than mere economic rationality.

The empirical findings obtained from the research area and discussed above in their cognitive, economic, and sociological dimensions are synthesized in Table 3 to reflect market dynamics from a more holistic perspective.

Upon examining Table 3, the structural contradictions and market realities on the dealer-producer axis are clearly summarized. The data in the table confirm that, on the one hand, a profound distrust towards institutional inspections and authorization processes (especially in Aksaray and Kırıkkale provinces at the 99% confidence level) dominates the market, while on the other hand, dealers have unwavering self-confidence regarding their own technical competences.

Furthermore, another critical analytical output revealed by Table 3 is the coexistence of regional fluctuations and invariable rules in producer behaviors. It was determined that dealer recommendation (4.45) and brand reliability (4.49) in purchasing decisions are overarching, invariable decision factors across provinces. However, when it comes to the level of producer awareness and actual product use, the homogeneous structure disappears. Table 3 clearly shows that producer awareness is statistically higher in Nevşehir and Niğde provinces and that liquid fertilizer usage trends are entirely integrated with the local crop pattern. The prominence of potatoes in Nevşehir and Niğde, corn in Aksaray, and cereals and legumes in Kırıkkale and Kırşehir proves the existence of product-specific approaches shaped according to micro-regional production dynamics, rather than a uniform marketing strategy in the liquid fertilizer market.

Consequently, Table 3 summarizes that supervisory weaknesses and the perception of high profitability go hand in hand in the market, presenting a structural breakdown showing that new strategies to be developed in the liquid fertilizer market should be constructed considering the local crop pattern, the establishment of institutional inspection mechanisms, and trust-based dealer-producer relationships.

Table 3: Key Findings on Dealer and Producer Dynamics in the Liquid Fertilizer Market

Assessment Area	Empirical Output and Dominant Trend	Prominent Regional and Statistical Differences
Market Inspection and Authorization	Inspection and authorization systems are found inadequate.	Distrust in the system is significantly high in Aksaray and Kırıkkale at the 99% confidence level.
Dealer Self-Efficacy	Complete self-confidence exists regarding technical knowledge and field experience.	There is no statistical difference among provinces in terms of the high competence perception of dealers.
Product Profitability and Impact	Products increase yield and offer high commercial profit margins.	According to the Kruskal-Wallis test, profitability perception is statistically more dominant in Kırıkkale, Aksaray, and Niğde.
Producer Awareness Level	Producers' awareness remains at a moderate level across the region.	Producers in Nevşehir and Niğde are significantly more conscious according to variance analysis (F=6.38).
Purchasing Decision Factors	Dealer recommendation (4.45) and brand reliability (4.49) are the most determining factors.	There is no statistical divergence ($p>0.05$) among provinces in purchasing criteria.
Product-Based Usage Trend	Usage intensity is shaped according to the pattern of regional agricultural products.	Potatoes in Nevşehir and Niğde, corn in Aksaray, and the cereal group and legumes in Kırıkkale and Kırşehir stand out.

CONCLUSION AND RECOMMENDATIONS

This research, conducted on 89 commercial fertilizer dealer enterprises operating in the TR71 Region, has empirically revealed the structural dynamics of the liquid organic and mineral fertilizer market, the institutional and commercial perceptions of dealers, and the decision-making mechanisms of final producers. Research findings indicate that the market is shaped not only by the balance of supply and demand but also by the dynamics of information asymmetry, a lack of institutional trust, and relationship marketing.

Findings regarding the institutional functioning of the sector point to a deep crisis of trust in dealership authorization processes and market inspection mechanisms. The fact that this distrust exhibits a statistical divergence at the 99% confidence level (χ^2=33.78), particularly in Aksaray and Kırıkkale provinces, proves the existence of a structural gap that paves the way for the spread of poor-quality (lemon) goods in the market. In these market conditions where institutional inspections are weak, dealers have come to the forefront with their technical expertise and field experience, rising to the position of primary technical authority (opinion leader) in the eyes of the producer. The homogeneous and high self-confidence of the dealers regarding their own technical competencies supports this authority building.

From the perspective of economic rationality, the perception that liquid fertilizers offer a high profit margin is prevalent across the region. The fact that this perception is statistically stronger (p=0.03) especially in Kırıkkale, Aksaray, and Niğde shows that macroeconomic regulations such as VAT exemption increase value capture in distribution channels rather than lowering producer costs. The fact that liquid fertilizers belong to an innovative input segment allows dealers to maximize their profitability by applying value-based pricing strategies.

When the behaviors of producers, who are the final consumers, are examined, a structure consistent with Technology Acceptance Models (TAM) is observed. Associating liquid fertilizer use directly with land scale rather than the producer's wealth level confirms that enterprises with economies of scale adopt innovative inputs more rapidly due to perceived usefulness, such as ease of application and time savings. However, cognitive dissonances, such as producers in Kırşehir province having high tendencies to read labels yet failing to accurately analyze plant needs, indicate serious barriers in converting

technical knowledge into actual agricultural practice (knowledge-action gap). At this point, the fact that the strongest factors determining purchasing decisions are dealer recommendation (4.45) and brand reliability (4.49) clearly reveals that the lack of knowledge and technical infrastructure is substituted by the trust placed in the dealer by the producer (relational benefits) and social capital. The clustering of product-based usage habits around potatoes in Nevşehir and Niğde, corn in Aksaray, and cereals-legumes in Kırıkkale and Kırşehir (path dependency) necessitates integrating marketing strategies into micro-regional crop patterns.

In light of all these findings, the following strategic recommendations are presented to enable the liquid organic and mineral fertilizer market in the TR71 Region to attain a sustainable, competitive, and reliable structure:

1. **Elimination of Information Asymmetry and Establishment of Institutional Trust:** o combat "under-the-counter" and counterfeit products that cause unfair competition in the market and shake producer trust, the inspection mechanisms of the Ministry of Agriculture and Forestry should be tightened. The effectiveness of the Fertilizer Tracking System (GTS) must be increased, and dealership authorization criteria must be reorganized based on merit and technical competence (preferably an agricultural engineering background). This way, the risk of "bad goods driving out good goods" (adverse selection) in the market should be eliminated.
2. **Balancing Profit Margins and Reducing Producer Costs:** Structural market regulations and price control mechanisms should be developed to ensure that supportive macroeconomic policies such as VAT exemption reflect directly as discounts on producer costs rather than expanding the profit margins of actors in the value chain.
3. **Overcoming "Cognitive Dissonances" in Producer Awareness:** To close the gap between producers' access to information (label reading) and turning information into practice (proper fertilization), practical extension and training activities should be increased under the coordination of Provincial and District Directorates of Agriculture and Forestry and chambers of agriculture. By strengthening soil test subsidies, the use of data-driven agricultural inputs should be encouraged.

4. **Development of Micro-Regional (Product-Specific) Strategies:** Based on the reality that liquid fertilizer use is strictly tied to the regional crop pattern, sector representatives should customize their marketing and sales strategies according to the specific product ranges of the provinces (e.g., potatoes in Nevşehir, corn in Aksaray).
5. **Institutionalization of Relationship Marketing:** Considering the reality that dealer recommendation and brand reliability are the main determinants in producer preferences, agricultural input companies should strengthen their local distribution networks and turn the social capital between "dealer and producer" into an institutional advantage by supporting the technical knowledge capacities of dealers with periodic training.

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