PRESENT INSIGHT IN NATURAL SCIENCES

EDITORS

Prof. Dr. Ahmet KAZANKAYA
Assist. Prof. Dr. Adnan DOĞAN

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PREFACE

Distinguished Readers,

Scientific inquiry into the natural world has always been driven by the need to understand, adapt, and innovate in the face of constant change. In today's world—marked by environmental instability, technological acceleration, and global challenges—the role of natural sciences has become more critical than ever. From climate change and biodiversity loss to sustainable agriculture and nanotechnological breakthroughs, the scope and impact of scientific research have expanded significantly.

This book, titled *Present Insight in Natural Sciences*, brings chapters authored by experts from various disciplines. The contributions reflect contemporary developments in life sciences, nanotechnology, environmental studies, and resource management, with a shared goal of offering innovative and sustainable solutions to natural science, agriculture, forest and fisheries science disciplines. Each chapter not only presents current scientific data and methodologies, but also sheds light on the broader ecological and societal implications of the research.

Our primary aim in compiling this book is to create a platform that bridges scientific advancement and practical application, while fostering a deeper understanding of how natural sciences can serve both humanity and the ecosystems we inhabit. We hope that this book will serve as a valuable resource for researchers, students, and professionals seeking to navigate the evolving landscape of natural sciences.

We would like to extend our sincere gratitude to the esteemed authors whose knowledge, vision, and dedication have enriched this work. We also thank the readers who engage with this book in pursuit of scientific progress and global sustainability.

Sincerely,

August 2025

Prof. Dr. Ahmet KAZANKAYA

Assist. Prof. Dr. Adnan DOĞAN

CHAPTER 1

USE OF MOLASSES IN ANIMAL FEEDING

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Sugar beet is normally a biennial plant, but can become an annual under certain conditions. In the first year, it produces a large, fleshy taproot and, in the second year, a flower stalk. Generally, sugar beet roots are planted in the spring and harvested in the fall of the same year. To produce seeds, the root requires a vernalization period at temperatures between 4 and 7 degrees Celsius (°C) to initiate bolting and begin the reproductive phase in the following growing season. During its first growing season, sugar beet has smooth, dark green, ovoid or heart-shaped leaves, forming a rosette from an underground stem. A white, swollen taproot develops at the junction with the stem. In the second growing season, the root produces a flower stalk that reaches a height of approximately 1.20 to 1.80 meters. A large petiolate leaf and smaller leaves appear at the base of the stem. Higher up the stem, the petiolate leaves become rarer, and then leaves appear whose blades are directly attached to the stem (sessile leaves). Lateral shoots develop from the leaf axils, forming a series of indeterminate clusters. The small sessile flowers can grow singly or in clusters. Sugar beet produces perfect flowers, with a three-headed pistil surrounded by five stamens and a perianth of five fine sepals. Petals are absent, and each flower is supported by a thin green bract. The ovary develops into a fruit, located at the base of the perianth, containing a single round or kidney-shaped seed. The common receptacle of the inflorescence protects the ovaries. A monogerm seed is produced when a single flower blooms, while a polygerm seed is produced by two or more flower clusters (Anonymous, 2017).

The presence of sugar in sugar beets was first observed in 1705 by Olivier de Serres, a prominent French agronomist, but this did not lead to immediate progress. It wasn't until 1747 that German chemist Andreas Marggraf demonstrated that the sugar crystals obtained from beet juice were identical to those from sugar cane. His student, Karl Achard, later developed an industrial process for extracting beet sugar, marking the beginning of sugar beet production in Europe. Extracting sucrose from sugar beets involves heating water to produce raw juice, which is then purified, filtered, and concentrated through several rinsing and evaporation stages. The final product is obtained by crystallizing the thick juice, producing white sugar, which is then recrystallized to produce high-quality refined sugar. Sugar beets are used in various stages of processing to make a variety of products. The by-product, mainly composed of water, contains up to 75% beet pulp, which serves as a heat source and can be reused in a closed circuit to meet a significant portion of the heat needs related to sugar production. After sucrose extraction, the remaining beet pulp and shavings are mainly used as animal feed or for biogas production. In addition, initiatives are underway to use beet leaves for methanol production. The molasses obtained after centrifugation of the thick syrup is mainly used for alcohol production, as animal feed, or as a growth medium for yeast biomass (Tomaszewska et al., 2018). Therefore, in this section, we present the use of molasses in animal nutrition.

1.TYPES OF MOLASSES

The Association of American Feed Control Officials (AAFCO, 1982) classifies the different types of molasses as follows:

- Cane molasses is a by-product of the extraction or refining of sucrose from sugarcane. It contains at least 46% total sugars, expressed as invert sugar. If the moisture content exceeds 27%, the density, measured by double dilution, must be at least 79.50 Brix.

- Beet molasses is produced by extracting sucrose from sugar beets and must have a total sugar content of at least 48% (also expressed as invert sugar) and a density of at least 79.50 Brix (measured by double dilution).

- Citrus molasses is the partially dehydrated juice obtained from dried citrus pulp. It must contain at least 45% total sugars, expressed as invert sugar, and a minimum density of 71.00 Brix.

- Hemicellulose extract is a by-product of wood processing. It consists of concentrated soluble substances obtained from wood treated at high temperature and pressure, without acids, alkalis, or salts. This extract contains pentose and hexose sugars and must have a total carbohydrate content of at least 55%.

- Starch molasses is a by-product of the production of dextrose from corn or sorghum starch by enzymatic and/or acid hydrolysis. It must contain at least 43% reducing sugars (expressed as dextrose), at least 50% total sugars (expressed as dextrose), and a total solids content of at least 73%.

2. MOLASSES IN TÜRKİYE

Türkiye has 33 sugar factories, including 15 private ones, 6 cooperatives, and 12 Türkşeker factories. According to the 2023 campaign data, there are

99,714 farmers, 3,628,146 hectares of arable land, and 25,250,213 tons of sugar beet. In the same year, 418,800 tons of molasses were produced in Türkşeker and 665,800 tons in the sugar industry. While Türkşeker's share in Turkish molasses production was 71% in 2002, it decreased to 49% due to changes in the sector's composition alongside production and quota volumes, and reached 39% in 2023, as shown in Table 1. In May 2023/24, a total of 1,190 tons of molasses were exported to Türkiye. It was observed that this was the strongest year for exports in the last five years. Molasses exports in 2021/22 were influenced by the amount of molasses produced and exports due to climate-related drought and low sugar beet yields. It was observed that import figures remained lower than export figures in the last two years (Türkşeker, 2023).

Table 1. Import, export and molasses production (Ton) (Türkşeker, 2023)

Years	Turksugar	Cooperative + Private Factories	Total	Import	Export
2019	283.600	438.607	722.207	442.500	261
2020	381.800	628.741	1.010.541	255.778	350
2021	278.190	425.967	704.157	410.424	257
2022	255.600	483.107	738.707	340.564	858
2023	418.800	665.800	1.084.600	153.990	1.190

3. OBTAINING MOLASSES

Molasses is the last remaining sugar syrup not recycled during the production of sugar beet and sugar cane in sugar factories. It is the basic raw material for the alcohol, yeast, and animal feed industries, and is also used for citric acid fermentation. Molasses accounts for 4% of the processed sugar beet. During the technical inspection of the Kırşehir Sugar Factory, the first author observed the following process (Figure 1):

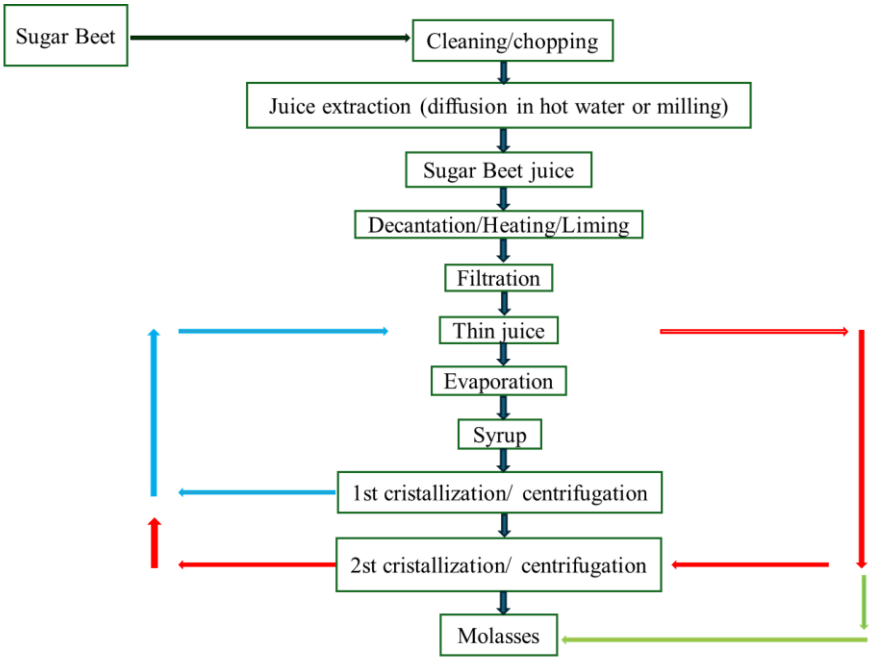

Figure 1. Obtaining molasses (personel observation by Fatih Şahan)

Molasses Production Steps: After weighing the sugar beets arriving at the factory, the washing process begins. This process removes soil, sludge, etc. from the sugar beets. The process of separating stones and grass is then repeated three times to ensure that the sugar beets are ready for cleaning at the factory. Once cleaning is complete, the sugar beets are transported to the factory. The first sugar beets to arrive are ground into finer pieces. The liquid portion is then extracted from the sugar beets by diffusion, and the remaining solid portion is stored for use as beet pulp. The extracted sugar liquor is mixed with lime water to separate the non-sugar components and inorganic matter, producing pure sugar water. This is then boiled and concentrated to form granulated sugar. The pure sugar water and granulated sugar are then separated by centrifugation to produce granulated sugar. The remaining pure sugar liquor is boiled again and centrifuged to obtain the second sugar. The remaining pure sugar liquids are stored as molasses.

4.CONTENT OF MOLASSES

Molasses is a thick, dark byproduct of processing sugarcane or sugar beet into sugar. It provides a quick source of energy and an excellent source of minerals for livestock. It also plays an important role in economical feed and pasture management. Sugarcane molasses is high in calcium (up to 1%) but low in phosphorus. Beet molasses, on the other hand, is higher in potassium and sodium but lower in calcium. It also contains significant amounts of trace minerals such as copper, zinc, iron, and manganese. Adding molasses to poor-quality hay can increase feed intake and improve flavor. Microorganisms in the rumen rapidly break down the sugar in molasses, resulting in a rapid release of energy. This makes molasses particularly effective for balancing different feedstuffs throughout the year in dairy cow diets. The use of molasses in animal nutrition promotes the digestion of hay and pasture, increases milk production, helps maintain body condition and appetite, and reduces feed waste. Cane sugar offers similar benefits to molasses and is an economical alternative. Molasses is composed of approximately 74% dry matter, 6.5% crude protein, and 65% sugars, providing 12.5 MJ of ME per kg of dry matter (Senthilkumar et al., 2016).

Table 2. Nutrient and mineral content of molasses (Mordenti et al., 2021)

Nutrients		Minerals			
Density, kg/L	1,39	CA, g/kg	0,7	Fe, g/kg	22,0
Dry Matter, g/kg	787	P, g/kg	0,5	Mn, g/kg	19,0
Ash, g/kg	90	Mg, g/kg	0,1	Zn, g/kg	13,0
Crude Protein, g/kg	98	K, g/kg	41,0	Cu, g/kg	7
Crude Fat, g/kg	2	Na, g/kg	7,2	Mo, g/kg	0,2
Gross Energy, kcal	2570	Cl, g/kg	4,3	I, g/kg	0,3

The value of molasses in animal feed is primarily due to its sugar content, which is approximately 50%. Compared to concentrated carbohydrates, molasses has a relatively low protein content but also contains small amounts of non-protein, non-sugar compounds that may have nutritional benefits, particularly for ruminants. It is generally advisable to add molasses to the diet when excess protein needs to be compensated. Although rich in minerals, molasses is generally lacking in calcium and phosphorus. These factors should be taken into account when formulating compound feeds, and appropriate feed supplements (such as lime) or a suitable feed combination should compensate for these deficiencies (Senthilkumar et al., 2016).

Table 3. Amino acid contents of molasses (Mordenti et al., 2021)

Sugar Beet Molasses			
Lysine, g/kg	0,5	Leucine, g/kg	1,7
Methionine, g/kg	0,3	Tyrosine, g/kg	1,6
Cysteine, g/kg	0,3	Valine, g/kg	1,1
Threonine, g/kg	0,7	Alanine, g/kg	2,2
Tryptophan, g/kg	0,2	Aspartate, g/kg	5,6
Isoleucine, g/kg	1,7	Glutamate, g/kg	36,0
Arginine, g/kg	0,3	Glycine, g/kg	1,8
Phenylalanine, g/kg	0,5	Proline, g/kg	0,9
Histidine, g/kg	0,2	Serine, g/kg	1,7

Molasses is accepted as an animal feed raw material due to its fermentability and high energy value. Its low cost makes it a valuable feed supplement. Rich in mineral salts, it offers an energy value half that of corn. The total sugar content of sugar beet molasses varies between 48 and 53%. As the sugar content increases, the osmotic balance deteriorates, affecting its shelf life. To extend its shelf life, it has been shown that the sugar content must be above 48% and the water content below 25%. Sugar beet molasses is evaluated based on its nitrogen content relative to crude protein (N x 6.25). Its nitrogen value is 8.7% higher than that of conventional feed. Crude protein content consists of free amino acids (glutamic acid, aspartic acid, leucine, tyrosine, arginine, and histidine) and nitrogen sources (betaine, guanine, and xanthine). These nitrogens present in molasses, like methionine, are known to play an important role in reducing fatty liver problems, which are common in dairy cows (Mordenti et al., 2021).

The vitamin content of molasses (Table 4) was low in fat-soluble vitamins. The thiamine content of water-soluble B vitamins was also low. Vitamin B1 deficiency is known to play an important role in glucose metabolism. Increased molasses supplementation significantly increases the vitamin content of the diet. Biotin is known to increase the risk of hoof disease and milk production in high-yielding dairy cows.

Table 4. Vitamin content of molasses (Mordenti et al., 2021)

Sugar Beet Molasses			
Vitamin A, mg/kg	-	Niacin, mg/kg	44,6
Vitamin E, mg/kg	2,4	Pantothenic Acid, mg/kg	4,58
Biotin, mg/kg	0,7	Riboflavin, mg/kg	2,2
Choline, mg/kg	890	Thiamine, mg/kg	1,0
Folic Acid, mg/kg	0,34	Pyridoxine, mg/kg	5,2

The ash content of sugar beet molasses is 8–9%. Rich in sodium and potassium ions, it helps balance the calcium and phosphorus intake of molasses added to the ration. The disadvantage of molasses lies in its content of potassium salts, which have a demineralizing and laxative effect. Its physical properties make it a preferred feed ingredient, offering numerous applications, both in feed mills and on farms. Furthermore, molasses has been observed to stimulate appetite due to its sweetening properties and increase feed consumption when added to feed ingredients that animals do not like due to their sweetening properties. The addition of molasses to feed ingredients also exhibits binding properties. It is particularly used in the production of pelleted feed (Mordenti et al., 2021).

5. USE OF MOLASSES IN ANIMAL NUTRITION

Molasses is mainly used in animal feed worldwide because it improves fiber digestion and non-protein nitrogen utilization by promoting microbial growth in the rumen of livestock. In addition, molasses is recognized for its health benefits due to its high mineral content (Bor-Sen et al., 2011). Due to its high content of nitrogen compounds, carbohydrates, and sweet taste, molasses finds many applications, both in the food industry and other fields. However, excessive use of molasses can cause neurological disorders such as incoordination and blindness in animals (Anonymous, 2024a).

Molasses can be effectively incorporated into the diets of all ruminants and is a cost-effective method of improving feed palatability while providing healthy levels of energy and protein. For dairy cows, it is recommended to include up to 3 kg of molasses per animal per day in complete feed formulations. For cattle, up to 10% molasses can be incorporated into the diet, depending on the type of other feed and the storage conditions of the finished feed. Similarly, young animals from four weeks of age can safely receive up to 10% molasses in their diet. For sheep, molasses can make up up to 10% of their dry ration, but it is generally not the preferred choice for block or lick feeders (Senthilkumar et al., 2016).

Molasses can be administered in various ways and in varying amounts. When administered directly, it is usually mixed with water. This diluted molasses is intended solely as a feed supplement and should not be used as drinking water. Ideally, the molasses solution should be poured or sprinkled over the feed in the trough or manger. The molasses adheres to the feed, preventing animals from selectively grazing. Once the mixture is evenly distributed, the daily amount of molasses can be accurately measured by diluting it in a container. Opinions differ regarding the appropriate water-to-molasses ratio. Ratios can range from 4 to 6:1, 2 to 4:1, or even 1:2. The most important requirement is that the solution mixes well with hay, chopped materials, and roughage. Small mechanical devices facilitate the mixing of diluted molasses with chopped straw. For silage, molasses that is as concentrated as possible and uniformly distributed is desirable. Uniform distribution can be achieved using a fan-driven chopper equipped with a small gear pump, provided the molasses is heated to about 70 °C or diluted 1:1 with water (Senthilkumar et al., 2016).

According to Senthilkumar et al. (2016), the use of molasses as livestock feed offers significant benefits:

- Cost-effective and high-quality nutrition
- Nutritional compensation for excess protein in green fodder through the addition of crude fiber
- Improved palatability of raw fodder and low-quality agricultural products
- Increased milk production and milk fat content
- Nutritional balance in a homogeneous and high-quality feed, preventing fertility problems during breeding
- Reduction of the dusty and powdery nature of some finely ground feeds by making the feed mixture more palatable and edible for livestock
- Replacement of missing sugars and trace elements and support of fermentation in low-quality feeds, particularly those with low sugar content
- Reduction of oat consumption in horse diets, thus reducing the risk of colic
- Reduction of dependence on cornmeal and other plant and grain feeds in pig diets
- Prevention and Treatment of certain deficiency diseases
- Filling gaps in the natural agricultural cycle: from field to livestock and back to field. This allows for more efficient use of fertilizers and ensures that important minerals, trace elements, and other substances essential for high yields are returned to the soil.

Pate et al. (1985) conducted a five-year study with 124 Brangus cows to evaluate the effects of weaning calves at 8.5 or 10.5 months and supplementing them with 2.25 kg of molasses per day during the winter. In late lactation, cows weaned at 10.5 months gained 14 kg less weight than those weaned earlier. Weaning age of calves had no effect on reproduction; however, calves weaned at 10.5 months were 2 to 3 days younger and weighed 5.3 kg less at 8.5 months. During the last two months of lactation, calves weaned at 10.5 months gained 37.2 kg and had a weaning weight 31.9 kg higher than calves weaned at 8.5 months. Molasses supplementation resulted in significant weight differences between cows. Molasses-fed cows had a calving rate 5 to 7 units higher than non-molasses-fed cows. Feeding molasses for 145 days, during the calving and nursery periods, resulted in an increase in weaning weight of calves at 8.5 and 10.5 months of age, by 7.7 kg and 11.2 kg, respectively, compared to non-molasses-fed cows.

Feroci and Nistri (1988) concluded that when dairy cows were fed molasses, dry matter intake, milk production, persistence of lactation curve and milk fat content increased and diseases such as live weight loss and ketosis decreased in dairy cows.

Keady (1996) reported in his study that the addition of molasses to wild grass during silage improved silage fermentation and increased feed intake, but had no effect on animal performance.

Murphy (1997) showed that the addition of molasses to the ration increases the palatability of the ration in cattle fed with poor quality grass and that there is an increase in dry matter intake because molasses has a stimulating effect on the digestive system.

Önol et al. (1998) reported that molasses added to straw (roughage of sheep) increased dry matter and crude protein intake, but had no effect on digestibility and rumen parameters.

Bailey and Welling (1999) conducted a study under grazing conditions in which dehydrated molasses blocks containing 30% crude protein, of which only 12% was provided by non-protein nitrogen compounds, 4% crude fat and 2.5% crude fibre increased pasture utilisation in cattle.

Ada et al. (2003) concluded that molasses sorbet up to 3% can be added to lamb fattening rations alone or with molasses instead of molasses.

In their study "Influence of hardener and molasses content of urea-melas lick blocks on block consumption," Ünal et al. (2004) fed lambs molasses blocks containing 30%, 35%, 40%, and 45% molasses. They found that the best results were obtained when mortar was used as a hardener in the preparation of urea-melas lick blocks, and that the daily block consumption by lambs increased with the molasses content.

Broderick et al. (2004) showed that adding molasses to the ration of dairy cows during the dry period increased roughage digestibility and prevented metabolic disorders such as ketosis that occur during the postpartum period.

Titgemeyer et al. (2004) investigated the influence of forage quality on the response of cattle to cooked molasses block supplementation. In Experiment 1, 175 heifers were given unrestricted access to meadow hay (5.2%

crude protein on a dry matter basis). Treatments were arranged in a 2 x 3 factorial design that included supplementation with 0 or 1.96 kg alfalfa dry matter/day and supplementation with no cooked molasses blocks or with a low- or high-protein cooked molasses block (14.4% or 27.5% crude protein on a dry matter basis, respectively). No significant interaction was observed between alfalfa and cooked molasses blocks on intake or weight gain. The addition of alfalfa resulted in increased feed intake and average daily gain. Heifers fed high-protein cooked molasses blocks showed greater weight gain than those fed low-protein cooked molasses blocks or those not fed. Heifers consuming high-protein cooked molasses blocks consumed more feed than those fed low-protein cooked molasses blocks, while heifers not fed molasses blocks fell between the two groups. Experiment 2 examined the effects of cooked molasses blocks containing 33% crude protein (dry matter basis) on 18 steers fed 1) bromegrass (8.4% crude protein), 2) alfalfa (19.2% crude protein), or 3) bromegrass with 1.93 kg/day of alfalfa dry matter. Their feed was available ad libitum. Forage dry matter intake was not affected by the cooked molasses block and was higher for alfalfa than for the alfalfa/bromegrass mixture, which was higher than for bromegrass. Dry matter digestibility was better for alfalfa than for bromegrass or the alfalfa/bromegrass mixture, and this was not affected by cooked molasses block supplementation. Supplementation with cooked molasses blocks had minimal effects on the intake and digestion of medium- to high-quality forages but improved weight gain and feed efficiency of heifers fed unrestricted meadow hay, whether or not they received supplemental alfalfa.

Polat et al. (2005) fed 40 male Merino lambs 3% and 6% molasses. They found that a 3% dose of molasses in the lambs' diet increased their live weight.

Bingöl et al. (2009) investigated the effects of adding molasses at different concentrations to barley and sainfoin silages on silage quality and digestibility. They concluded that adding molasses to silage composed of equal amounts of barley and sainfoin, specifically at concentrations of 4% and 6%, improves silage quality and digestibility in both forms, resulting in low ammonia nitrogen and high lactic acid content. It also promotes the carbohydrate content of crops with difficult silage properties, such as sainfoin, and, combined with barley, results in suitable silage.

Bingöl et al. (2010) concluded that silage with 5% molasses added to Jerusalem artichoke silage has a positive effect on organic matter digestibility and in vitro fermentation parameters and that Jerusalem artichoke silage can be used as an alternative feed source for animals by silage with or without additives.

Shotorkhoft et al. (2013) added molasses instead of corn/barley as an energy source to heat-treated broiler meat diets and concluded that the diet increased dry matter (DM) and crude protein (CP) content and decreased pH.

Konca et al. (2015) found that the addition of molasses to sunflower silage positively affects silage quality.

Adding 3% molasses to the diet results in improved microbial activity, increased protein synthesis, and reduced ammonia content, followed by a reduction in the amount of urea in blood and milk (Brito et al., 2015).

Gordon et al. (2016) reported that adding molasses-based liquid feed to the high-grain calf ration could reduce changes in calf feed intake.

de Ondarza et al. (2017) reported that adding high amounts of molasses (10%) to the diet can cause metabolic diseases and recommended administering raw materials containing between 6 and 22% cellulose in combination with molasses as a preventative measure. They indicated that the daily intake of molasses should be limited to 1 kg per animal per day for cattle, 0.5 kg per animal per day for heifers, and 1.0 to 1.5 kg per animal per day for dairy cows. They concluded that the molasses content of the dry matter in the diet should not exceed 6.75%.

In their study on the use of acorn silage supplemented with molasses in lamb fattening, Kurt et al. (2017) found that up to 30% molasses can be used in acorn silage depending on the dry matter.

In their study on the quality of black-eyed pea and soybean silages supplemented with molasses or barley semolina, Gülümser et al. (2019) found that adding 10% molasses to silages increased the in vitro digestibility of nutrients.

Daş et al. (2019) concluded that Lenox plant can be ensiled by adding 7% and 10% wheat straw and 1%, 2% and 3% molasses, and the resulting silages generally have the quality of high-quality silage and can be used as an alternative source of roughage in ruminant feeding.

Havekes et al. (2020) reported that adding molasses-based liquid feed to a high-straw ration increased nutrient intake and protected rumen health in dairy cows during the dry period and early lactation.

Osman et al. (2020) reported that adding 5–40% molasses to goat diet improved animal health, immunity, growth performance, protein metabolism, and rumen fermentation.

Şahin et al. (2020) concluded that the addition of up to 4% molasses to Italian ryegrass silages resulted in improvements in nutrient and silage quality measures.

Bolakar et al. (2021) concluded that 4% molasses can be added to Phyllotamus (Miscanthus x giganteus) silages with different urea and molasses contents.

According to Mordenti (2021), molasses or molasses-based liquid feeds are used in dairy cows because they are inexpensive and easy to store, increase dry matter intake, increase biotin content, and reduce milk urea content. They are recommended to improve the performance of fattening animals.

de Nazaré Santos Torres et al. (2023) reported in their study that adding 100 to 150 g/kg of fat to the ration had no effect on carcass quality, but after adding 200 g/kg, daily live weight gain and carcass weight decreased.

In sheep, it helps increase dry matter intake before lambing. Adding molasses to the ration promotes cellulose digestion. Adding small amounts of molasses to the ration results in increased feed intake in lambs shortly before lambing (Anonymous, 2024b).

Molasses has been shown to be safe for use in poultry with positive results in geese, ducks, broiler chickens, and laying hens (Anonymous, 2024c).

It has been recommended to add 1 to 3 kg of molasses to rations, 1 to 2 kg for dairy cows, 0.2 to 0.5 kg for cattle and 0.2 to 0.5 kg for sheep (Anonymous, 2024d).

Easy to store, transport, and use, feed blocks provided a slow, continuous supply of nutrients when grazing was insufficient. The first feed blocks were used in ruminants and were found to optimize digestion, increase daily weight gain, and milk production. Blocks containing molasses have also been shown to be effective in treating common parasitic diseases in animals and increasing productivity. They are also used to increase feed intake (Anonymous, 2024e).

Mordenti et al. (2021) described the role of molasses in cattle nutrition in their review article as follows: (a) improved net energy and digestible protein availability; (b) increased dry matter intake, milk production, lactation curve persistence, and milk fat content; (c) improved fiber digestion and nutrient efficiency; (d) reduced risk of subclinical acidosis, rumen subacidosis, mucosal damage, and innate immune activation; and (e) reduced weight loss in lactating animals and reduced ketosis rates. It is important to limit daily molasses intake to less than 10% (6.75% of diet dry matter). In practice, it is recommended not to exceed 1 kg per animal per day for cattle, and for heifers, dry and lactating cows, the recommended quantities are respectively 0.5, 1 and 1.5 kg per animal per day.

According to Senthilkumar et al. (2016), uncontrolled administration of molasses can lead to toxicity; therefore, it is advisable to offer it in a controlled manner. When molasses is used as a supplement (often in combination with urea) or as a primary feed ingredient, three metabolic disorders can occur in cattle and sheep: urea toxicity, molasses toxicity, and bloat.

Urea toxicity occurs when there is unlimited access to molasses and urea mixtures. Urea consumption can potentially reach 300 g/day (e.g., a 500 kg dairy cow consuming 10 kg/day of the mixture). However, in these cases, the risk of urea toxicity is quite low, as the sugars and ammonia in the molasses are quickly incorporated into the development of microbial cells. Animals that have not previously been exposed to urea can safely consume molasses mixtures containing up to 3% urea without risk of toxicity. The reason for using molasses containing 8–10% urea is that the increased urea concentration prevents excessive consumption of the mixture. Toxicity only occurs if the urea is poorly mixed or if the high moisture content of the mixture encourages the animal to “drink” the mixture rather than “lick” it. To prevent urea toxicity, since molasses does not contain sufficient protein, 3 to 4 kg of urea can be added to 100 kg of molasses, but the mixture must be mixed well (Futurebeef, 2011).

Molasses poisoning was once the most significant risk associated with unrestricted molasses feeding. For example, after the introduction of the molasses-urea feeding system in Cuba, mortality and emergency slaughter rates on a 10,000-head feedlot operation increased from 0.1% and 0.4% (with feedlot feeding) to 1% and 3%, respectively, after switching to a high-molasses and high-urea diet. Molasses-poisoned cattle exhibit excessive salivation, an isolated, dejected posture, often with their heads down, and are commonly backed against gates or feed bunks. They often suffer from impaired vision, possibly leading to blindness. When disturbed, their movements become unsteady and uncoordinated. The nervous system symptoms and blindness characteristic of molasses poisoning indicate brain lesions clinically indistinguishable from cerebrocortical necrosis (CCN), also known as polioencephalomalacia (Edwin et al., 1979). Visible cerebral necrosis allows for a rapid diagnosis. This necrosis is probably due to a decrease in cerebral energy supply, either due to a total deficiency of thiamine in the diet, to the uptake of thiamine analogues in the rumen and/or to the action of thiaminase in the rumen (Edwin et al., 1979), or to a glucose deficiency (Losada and Preston, 1973).

Bloat, the accumulation of gas in the rumen, either free or trapped in foam, can occur in almost any feeding system. It is more common in diets containing other low-fiber but easily digestible carbohydrate sources, such as raw sugar (MacLeod et al., 1968) and corn grains (Fermin et al., 1984).

To treat and prevent high-molasses feeding systems, it is common to limit the amount of forage provided (either to encourage molasses consumption or because it is more expensive than molasses). Forage deficiencies, whether quantitative or qualitative, appear to be the main factors contributing to molasses toxicity. Consequently, the incidence of molasses toxicity was lower when wheat or barley straw was used as a forage source than when sorghum or corn silage was used in molasses feedlots. Furthermore, no signs of toxicity were observed when using protein-rich forages such as Leucaena, cassava leaves, and sweet potato. Similarly, providing palatable, protein-rich forage to affected animals appears to be the most effective treatment method. Recent advances in molasses-based feeding have highlighted the technical and economic advantages of incorporating protein-rich forage crops, including legumes such as Leucaena, Gliricidia, and Erythrina, as a dual source of "fiber" and "bypass protein" (Preston et al., 1967). These methods are also likely a cost-effective approach to addressing molasses toxicity. The preceding discussion highlights the critical importance of management in any feeding strategy when economic constraints dictate suboptimal supplementation levels (Senthilkumar et al., 2016).

6. OTHER AREAS OF USE

In road maintenance, molasses is used to (i) reduce dust on roadways around sugar factories (Ndegwa, 2011) and (ii) produce molasses-derived materials for road de-icing (Sarka et al., 2012).

Tiwari et al. (1990) reported that the combination of molasses and urea nitrogen can help to stabilize rumen pH, improve dry matter digestion and regulate digestive function by stimulating the activity of rumen microflora.

Özyuğuran et al. (2016) showed in their study titled "Briquetting of Afşin-Elbistan Lignite with Addition of Biomass and Binder" that the addition of molasses, linobride and sulfuric acid as briquette binders increased the water resistance of briquettes.

Çelik et al. (2019) found that adding 10% or 15% molasses can increase the drop resistance during briquette production.

Özdiz et al. (2022) and Şanlı et al. (2015) found that molasses can be used both as a fertilizer and as a solution for plant nutrients. For example, sugar content and gross sugar yield increased significantly by 1.2% and 2.9 t/ha, respectively, after molasses treatment. This suggests that sugar beet molasses can be effectively used to increase sugar beet yield and quality (Şanlı et al., 2025).

Demirci (2025) found that molasses positively increased the refractive index of briquettes.

7. CONCLUSION

The importance of molasses in animal feed is therefore as follows:

- Easily digestible fiber: Adding molasses to rations improves fiber digestibility.
- Reduced feed loss and dust formation due to palletizing are avoided, thus improving animal body condition and appetite. Adding molasses to rations helps reduce feed loss.
- Molasses is an energy-rich feed supplement and is considered an energy feed due to its easily fermentable sugar content. It is a very valuable raw material when grain is scarce or expensive.
- Adding molasses to rations is known to improve growth and protein metabolism without compromising animal health.
- Minerals and nutrients: Molasses contains many minerals and nutrients. Rich in iron, calcium, magnesium, vitamin B6, selenium, and other vitamins, it is used as a healthy and balanced feed.

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CHAPTER 2

A WATER QUALITY CRITERIA, ARSENIC

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1. INTRODUCTION

Water, a colorless, odorless, tasteless, and transparent liquid composed of two hydrogen and one oxygen atom, which holds great importance both socially and economically, is a substance that covers approximately 75% of the Earth's surface and forms the hydrosphere, with the majority found in oceans and seas, and only a small portion existing as freshwater in lakes, rivers, and underground waters. Water is one of the most valuable resources of nature, indispensable for the life of both humans and all other living beings, necessary for basic biological functions such as nutrition, circulation, and excretion, and forming the habitat for many organisms. It regulates the climate on a global scale and provides routes for transportation. For these reasons, great civilizations throughout history have been established around water resources or have transported water to their settlements (Şen 2016; Bhat et al. 2023; Yazman et al. 2024).

Although water is abundant on Earth, the amount of usable freshwater is quite limited. Due to population growth, urbanization, industrialization, and environmental pollution, ecological balances have been disrupted, and it has become a serious problem that threatens human life. Effective water management requires preserving both the quantity and quality of water. Both surface water and groundwater are major sources for drinking water supply worldwide. Especially in fields such as agriculture, industry, and energy production, the conscious use of water is of vital importance. Approximately 43% of the water used in agricultural activities originates from groundwater sources. In addition, groundwater is also an important source for domestic use in many regions. However, industrialization, agricultural activities, urbanization, and other human activities cause water pollution. Water pollution, which is the adverse change in the physical, chemical, or biological properties of water, restricts the use of water resources and harms human health and biological life. Domestic and industrial waste, the use of agricultural pesticides and fertilizers, tourism activities, and climate change are the main factors causing water pollution (Gündüz 2009; Şen 2016; Bhat et al. 2023; Hassan 2023; Yazman et al. 2024).

As of 2021, more than 2 billion people worldwide live-in countries experiencing water stress. It is estimated that over 663 million people lack access to clean drinking water, and around 1.8 billion people consume drinking water contaminated with fecal matter. Microbiologically contaminated drinking water can facilitate the spread of infectious diseases such as diarrhea, cholera, dysentery, typhoid, and poliomyelitis. This situation is estimated to cause approximately 505,000 diarrhea-related deaths each year (Hassan 2023; WHO 2023).

Among the pollutants detected in water resources, arsenic stands out as a primary and serious threat. It can enter groundwater through natural processes as well as anthropogenic sources such as industrial waste, agricultural pesticides, domestic wastewater, and urban sewage. Due to its high solubility in water, it can be transported to rivers, lakes, and other water bodies through surface runoff. Therefore, drinking water is the major source of human exposure to arsenic. Its proven carcinogenic effects on human health make it a matter of global concern. Arsenic-related health problems have been reported in many countries, including Bangladesh, India, China, and the United States. It has been associated with gastrointestinal cancers, skin diseases, vascular disorders, diabetes, and heart conditions (Villaescusa and Bollinger, 2008; Vaclavikova et al. 2008; Alpaslan et al. 2010; Kumari et al. 2017; Bakan 2019; Yatkın 2021).

2. WHAT IS ARSENIC?

Arsenic has become almost synonymous with the word "poison" among the general public. It is a highly toxic metalloid element with properties intermediate between metals and nonmetals. Arsenic is commonly found in nature-within air, water, soil, rocks, and living organisms-through both natural processes and human activities. Due to its toxic effects, it is considered a major environmental pollutant that poses a threat to both public health and marine life (Jones 2007; Basu et al. 2014; Atabey, 2015; Kumari et al., 2017; Yatkın 2021). Arsenic has been known since the 17th century and is classified as a carcinogen by the International Agency for Research on Cancer (IARC) (Abdul et al. 2015).

Arsenic is the 20th most abundant natural element, ranking 14th in seawater and 12th in the human body. It occurs in elemental, organic, inorganic, and gaseous (arsine) forms, and is a major component of over 200 minerals, including elemental arsenic, arsenides, sulfides, oxides, arsenates, and arsenites. However, most of these arsenic-containing minerals are rare in nature and are typically found in ore zones as sulfides alongside gold, copper, lead, zinc, tin, nickel, and cobalt. The most commonly occurring arsenic minerals in the environment are Arsenopyrite (FeAsS), Realgar (AsS), and Orpiment (As_2S_3) (Vaclavikova et al. 2008; Başkan and Pala, 2009; He and Charlet 2013; Yatkın 2021; Hassan 2023). Arsenic is produced in countries such as Belgium, Chile, China, Morocco, and Russia (Atabey 2015).

2.1. The Chemistry of Arsenic

Arsenic, reported to have been discovered in 1250 by the German scientist Albertus Magnus, belongs to Group 5A of the periodic table and exhibits properties between phosphorus and antimony. Its chemical symbol is As, atomic number 33, atomic weight 74.91 g/cm³, and specific gravity 5.73 g/cm³. It is an odorless, colorless, solid, brittle, silver-colored element with a metallic luster. In nature, it is rarely found in its pure elemental form. It is resistant to air, water, acids, and bases. Arsenic has no liquid phase and evaporates through sublimation. Its sublimation point is 615 °C (it transitions directly from solid to gas), and its melting point is 817 °C (Garelick et al. 2008; Kumari et al. 2017; Atabey 2019; Buzoğlu 2019; Yatkın 2021; Genchi et al. 2022). Its biological half-life ranges from 10 hours to 1-2 days (Güler and Çobanoğlu 1997).

Arsenic exists in four main forms that elemental, gaseous, inorganic, and organic and in four primary oxidation states that +5, +3, 0, and -3. These correspond to As^{+5} (arsenate, As(V)), As^{+3} (arsenite, As (III)), As^0 (elemental arsenic), and As^{-3} (gas, arsine). In nature, the most common inorganic species are arsenate and arsenite, while the main organic species are the methylated forms of these compounds: monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA). Elemental arsenic is insoluble in water; however, its solubility in compound form varies depending on the chemical properties of the environment. The gaseous form is the most toxic. Inorganic arsenic compounds are more toxic than organic ones. Arsenite is approximately 60 times more toxic and more mobile than arsenate. Under anoxic conditions, arsenic trioxide (As_2O_3) is dominant and, when dissolved in water, forms arsenious acid (H_3AsO_2). In basic environments, it forms arsenite salts. These white arsenite salts are highly toxic; a dose of 0.06 to 0.2 grams can be lethal to humans (Leist et al. 2000; Jones 2007; Basu et al. 2014; Shakoor et al. 2016;

Bhat et al. 2023; Hassan 2023).

Arsenic occurs in nature in different allotropes (different structural forms of the same element) and can appear in white, gray, blue, yellow, red, and black colors. **Gray arsenic** is the most common form found in nature and the most widely used in industry. Elemental arsenic As^0, also known as metallic arsenic, is brittle and has a density of 5.73 g/cm³. It tarnishes when exposed to air (Figure 1). **Yellow arsenic** (As_2S_3) is the most volatile, least dense, and most toxic form. It has a soft, waxy texture and a density of 1.97 g/cm³. Under the influence of light, it rapidly transforms into gray arsenic (Figure 1). **Black arsenic** has a shiny, glass-like, and brittle appearance. It forms by cooling arsenic vapor at 150-220 °C or through crystallization in the presence of mercury vapor. It is stable in dry air, but in humid environments, a golden-bronze film forms on its surface, and it turns black over time. When heated in air, arsenic releases fumes with a garlic-like odor. Other commonly encountered forms include arsenic disulfide (**red arsenic**, As_2S_2) (Figure 1) and arsenic trioxide (**white arsenic**, As_2O_3) (Atabey, 2015; Atabey, 2019; Buzoğlu 2019; Yatkın 2021; Genchi et al. 2022).

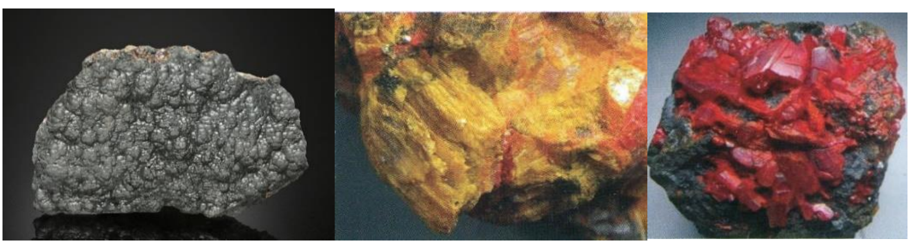

Figure 1. Gray, Yellow and Red arsenic (tr.geologyscience.com; Atabey 2015)

2.2. Main Uses Areas of Arsenic

Arsenic is an element that has been used throughout history in various fields, although its use has been relatively restricted after its harmful effects were recognized (Yatkın 2021; Genchi et al. 2022). Some of its uses are listed;

a). Historical use: During the Bronze Age, arsenic was added to bronze alloys to increase their hardness (Genchi et al. 2022).

b). Warfare technology: Due to its irritating effects on the skin, eyes, and respiratory system, some arsenic compounds have been used as chemical warfare agents. In World War I, lewisite was developed as a lung-irritating gas, while adamsite, a yellow, odorless gas, caused vomiting, sneezing, headaches, and nervous fatigue. During the Vietnam War, the United States used Agent Blue, an arsenic-based herbicide (Yatkın 2021; Genchi et al. 2022).

c). Medicine and pharmacy: In the past, arsenic compounds such as arsphenamine (Salvarsan) and neosalvarsan were used to treat syphilis, now treated with penicillin. Arsenic-based drugs like melarsoprol are still used to treat African sleeping sickness. In 2018, arsenic nanoparticles synthesized from arsenic trioxide were shown to kill breast cancer cells. Radioactive isotopes such as As-72, As-74, and As-76 are used for diagnostic purposes (Yatkın 2021; Genchi et al. 2022).

d). Agricultural use: Calcium and lead arsenates were used as pesticides from the late 1800s until the 1940s, before DDT was introduced. Paris green (copper acetoarsenite) and Scheele's green (copper arsenite) were used both as pigments and insecticides (Leist et al. 2000; Yatkın 2021; Genchi et al. 2022).

e). Pigment production: Arsenic sulfide compounds such as As_4S_3, As_4S_4 (red arsenic), As_2S_3 (yellow arsenic), and As_2S_5 were widely used as pigments, especially in the past. Scheele's green was even used as a food dye in certain sweets (Yatkın 2021; Genchi et al. 2022).

f). Industrial applications: Arsenic has been used in printing and textile dyes, leather and wood preservation and coating, lead batteries, semiconductors in electronics, metallurgy, ceramics and glass industries, ammunition alloys, and the production of bullets and fireworks, as well as an additive in bronze plating and heat-resistant alloys (Leist et al. 2000; Sarıhan 2013; Yatkın 2021; Genchi et al. 2022).

3. RESOURCES OF ARSENIC IN WATWER

Arsenic is transported to aquatic environments through both natural processes such as volcanic activity and rock weathering, as well as anthropogenic pollution. Arsenic levels in groundwater sources are generally higher than those in surface waters. In many coastal and delta regions around the world, arsenic contamination is increasing day by day. Studies have shown that arsenic accumulates in marine organisms and sediments mainly in organic form, while its conversion to inorganic form remains relatively low (Vaclavikova et al. 2008; Garelick et al. 2008; Bakan 2019; Yatkın 2021).

3.1. Natural Resources

Arsenic is a naturally occurring element found in rocks, soil, water, air, plants, and animals. The total amount of arsenic in the Earth's crust is estimated to be 4.01 million tons, with an average concentration of 6 mg/kg. In addition, natural processes such as seawater, volcanic activity, hot springs, geothermal processes, metamorphic rocks and mineral deposits, soil formation, dust storms, forest fires, coal combustion, and biological and microbiological activity contribute to arsenic release into the environment. Arsenic concentrations range from 0.09 to 24 µg/L in seawater and from 0.15 to 0.45 µg/L in surface waters. The most significant natural sources of arsenic include sulfur minerals (such as pyrite) and arsenic-bearing iron oxides, as well as lead- and copper-containing ores. Arsenic occurs in more than 200 mineral types, typically in sulfide form, in sedimentary and volcanic rocks and soils. Major arsenic-containing minerals include Orpiment (As_2S_3), Realgar (AsS), Arsenopyrite (FeAsS), Löllingite ($FeAs_2$), Gersdorffite (NiAs), Cobaltite (CoAsS), Enargite ($Cu_{12}As_4S_{13}$), and Tennantite (Cu_3AsS_4). Arsenic may also be present within iron oxides. These minerals are often concentrated in areas enriched with elements such as cadmium, lead, gold, silver, antimony, and molybdenum (Garelick et al. 2008; Vaclavikova et al. 2008; He and Charlet 2013; Shakoor et al. 2016; Yatkın 2021; Genchi et al. 2022).

Geothermal systems are rich in arsenic, with concentrations in these waters ranging from 0.1 to 50 mg/L. Arsenic is most commonly found within pyrite or bound to iron oxides. When hot water reaches the surface, arsenic in the form of As(III) is gradually oxidized to As(V). Mixing of these geothermal waters with groundwater or surface water can lead to elevated arsenic levels. In particular, the discharge of wastewater from geothermal energy production into the environment can contaminate both groundwater and surface water sources (Garelick et al. 2008).

3.2. Anthropogenic Sources

Arsenic is released into the environment not only through natural processes but also as a result of human activities. Humans have used arsenic in many fields and have produced and continue to produce various wastes. These fields include industry, agriculture, medicine and pharmacy, mining, warfare technologies, and pigment production. Industrial uses include the cosmetics industry, leather processing, cement production, paper industry, rubber production, the production of explosives and chemical weapons, the manufacture of devices such as transistors with no moving parts, and the production of lasers, light-emitting diodes (LEDs), and semiconductors. Arsenic pollution from mining varies depending on the type of mine. According to studies, the lowest arsenic concentration has been measured in iron mines, and the highest in lead-zinc mines. Arsenic can be released during the mining of metals such as copper, nickel, and gold, petroleum extraction, and ore processing. These pollute soil, marine, and freshwater aquifers. The raw phosphate rocks used in the production of phosphate fertilizers in agriculture contain high levels of arsenic (more than 50 mg/kg) and cadmium (Cd) (Sönmez et al. 2008). The use of pesticides can significantly increase arsenic levels in the environment. Common examples of arsenic-containing pesticides include lead arsenate, zinc arsenate, calcium arsenate, magnesium arsenate, and also aluminum phosphide (fumigant) and copper oxychloride (fungicide), which are among the licensed pesticides in Türkiye. Fossil fuel use and leachate from landfills, petroleum refining, coal combustion, and domestic waste incineration can cause arsenic pollution. Arsenic and its isotopes are also used in drug production and in diagnosis and treatment in medicine (Güler and Çobanoğlu 1997; Garelick et al. 2008; Vaclavikova et al. 2008; Başkan and Pala, 2009; Alpaslan et al. 2010; He and Charlet 2013; Sarıhan 2013; Atabey 2015; Shakoor et al. 2016; Buzoğlu 2019; Yatkın 2021).

3.3. Mobility of Arsenic

Among metalloids, arsenic is the most prone to becoming mobile within a pH range of 6.5-8.5 and under both oxidizing and reducing conditions. Biological processes, geochemical reactions, volcanic emissions, and various anthropogenic activities all contribute to arsenic mobilization. Under natural conditions, arsenic mobilization is the primary cause of environmental arsenic problems. However, mining activities, fossil fuel combustion, arsenic-containing insecticides, herbicides, agricultural desiccants, and feed additives for livestock also contribute to arsenic pollution. Arsenic is primarily transported through water in the environment (Hassan 2023). Under suitable environmental conditions, microorganisms can transform arsenic compounds into more complex organic arsenic species through biomethylation. These organic compounds may become widespread in water, particularly under the influence of agricultural and biological activities (Vaclavikova et al. 2008).

a). Geogenic (Natural) Factors: The main processes responsible for the arsenic concentrations observed in surface and groundwater are as follows (He and Charlet 2013).

Evaporation: In arid or semi-arid regions, excessive use of groundwater due to surface water scarcity, combined with high evaporation rates and low recharge, leads to increased arsenic concentrations, especially in low-lying areas and closed basins.

High pH: Adsorption and desorption reactions between arsenic and iron, manganese, and aluminum oxides control arsenic mobility in the hydrogeological environment due to the widespread presence of these oxides. Arsenate (As^{5+}) binds strongly to the surfaces of these oxides in acidic and near-neutral pH waters, whereas arsenate adsorption rapidly decreases in alkaline conditions.

Geothermal Effects: Arsenic release from geothermal systems into surface and groundwater has been reported in various parts of the world. Arsenic concentrations in waters from geothermal sources may reach 10 mg/L, 20 mg/L, or even higher. Hot springs, geysers, and steam vents discharge their arsenic-rich contents directly into nearby water basins, contaminating surface aquifer systems.

Sulfur Oxidation: Arsenic-bearing minerals, particularly arsenic-rich pyrite, arsenopyrite, orpiment, realgar, and arsenic-associated metal sulfides, may release significant amounts of arsenic due to changes in the chemical environment. Oxygen-rich conditions cause oxidation of these minerals, followed by arsenic mobilization.

Anaerobic Dissolution: Anaerobic microbial respiration is an important process that contributes to arsenic mobilization from arsenic-containing minerals, particularly hydrous iron oxides, by utilizing both sedimentary and surface-derived organic carbon.

b). Anthropogenic Factors: Arsenic may enter natural waters as a byproduct of certain human activities. These include chemical industry, mining operations, and agriculture. Arsenic-based pesticides, industrial wastewater/ sludge, and mine wastes contaminate waters through natural processes as well as direct human influences such as rainwater infiltration, irrigation, surface runoff, and sewage discharge (He and Charlet 2013).

4. HEALTH EFFECTS OF ARSENIC

Arsenic toxicity is a serious public health problem on a global scale, and this issue has reached critical levels particularly through the consumption of drinking water. Arsenic can enter the human body via water, food, soil, and air. For this reason, the World Health Organization, the EU, the USA, and countries like Turkey have lowered the arsenic limit in drinking water from 50 µg/L to 10 µg/L. In the past, arsenic was used in agriculture and as a wood preservative, and in medicine for the treatment of diseases such as syphilis and sleeping sickness; however, it is rarely used today (Villaescusa and Bollinger, 2008; Basu et al. 2014; Shakoor et al. 2016).

Arsenic toxicity is closely related to the form in which arsenic is present. Since the specific types of arsenic in the environment are often not identified, the extent and nature of human arsenic exposure may not be clearly known. Approximately 90% of inorganic arsenic salts in water are absorbed from the intestines into the bloodstream. They are then transported to the liver, circulated through the body, and distributed to different tissues. The liver, lungs, skin, nails, hair, and skeletal system are more prone to arsenic accumulation. The concentration of arsenic in muscle tissue is low. While a portion accumulates in tissues, most is excreted through urine. Inorganic arsenate (As+5) is eliminated at three different rates: 66% with a half-life of approximately 2.1 days, 30% with 9.5 days, and 3.7% with a 38-day half-life. It can replace phosphate and settle in bone, where it may remain for about 5000 days. Arsenite is generally excreted more rapidly. The average half-life of organic arsenic compounds is about 20 hours (Caussy and Priest, 2008; Basu et al. 2014; Abdul et al. 2015).

As^{5+}, which also affects cellular metabolism in multiple ways, inhibits energy production by replacing phosphate in ATP synthesis; whereas As^{3+} binds to thiol groups in proteins, causing structural damage. Arsenic can suppress antioxidant defense systems, leading to damage in DNA and cellular components and potentially causing cancer. Arsenolipids may disrupt growth functions by altering the structure and fluidity of the cell membrane. They have been shown to exert cytotoxic effects in human liver and bladder cells. In individuals who consumed cod liver containing arsenolipids, these compounds were found to be rapidly metabolized and excreted in the urine as dimethylarsinic acid and other metabolites, indicating their conversion into water-soluble forms for excretion (Bakan 2019).

Soluble forms of this element in water can accumulate in aquatic organisms and cause metabolic and physiological disorders. A study on *Oncorhynchus mykiss* showed that arsenic trioxide caused liver damage, osmoregulation disorders, and lipid peroxidation. In *Clarias gariepinus*, it was also reported to cause liver damage (Bakan 2019).

4.1. Arsenic Exposure

Arsenic is a toxic and carcinogenic element for many living species, including humans, and has historically been known to cause acute poisoning. However, from the early 20th century onward, the chronic effects of arsenic exposure also began to be recognized. The most common sources of arsenic exposure include drinking water and food contaminated with arsenic, air, and occupational environments (Caussy and Priest, 2008; Villaescusa and Bollinger, 2008; Başkan and Pala, 2009; Abdul et al. 2015; Genchi et al. 2022; Hassan 2023). Cigarette smoke also contains arsenic (Atabey 2015). Arsenic may also be deliberately ingested in cases of suicide, homicide, or crimes against humanity (Buzoğlu 2019).

The chemical form of arsenic affects its absorption. Elemental arsenic is poorly absorbed, whereas trivalent and pentavalent inorganic arsenic compounds are readily absorbed (Güler and Çobanoğlu 1997). Organic arsenic is also well absorbed. It can cross the placenta to reach the fetus. While inorganic arsenic species are health hazards, organic forms found in seafood are generally harmless (Abdul et al. 2015).

Chronic exposure to arsenic can lead to numerous health issues, including cancer, skin discoloration (melanosis), hyperkeratosis (thickening of the skin), Blackfoot disease, neurological problems, fatigue, numbness, respiratory disorders, immune suppression, gangrene, diabetes, anemia, ulcers, hypertension, heart disease, miscarriage, stillbirth, and premature births (Başkan and Pala, 2009; Yatkın 2021). For these reasons, arsenic is classified as a carcinogenic and mutagenic substance. In the human body, arsenic accumulates especially in keratin-rich tissues (hair, nails, skin), where signs of chronic exposure (hyperpigmentation, hyperkeratosis) appear. Men are more susceptible than women to arsenic-induced skin lesions (Villaescusa and Bollinger, 2008; Yatkın 2021). Arsenic is primarily excreted in urine. Inorganic arsenic remains in the body longer (Abdul et al. 2015).

4.2. Arsenic Toxicity

Arsenic is a colorless and odorless toxic substance that enters the human body through the digestive system, respiratory system, and parenteral (non-oral) routes. Its degree of toxicity depends on the form of the arsenic compounds, route of entry, amount and duration of exposure, age, and sex. The most toxic form of arsenic is arsine (AsH_3), while the least toxic are organic arsenic species, as they are easily excreted and have minimal health effects. In general, trivalent compounds are ten times more toxic and 4 to 10 times more water-soluble than pentavalent ones, and up to 70 times more toxic than the organic arsenic species MMA(V) and DMA(V). Arsenic inhibits enzyme function by binding to thiol groups in enzymes. Arsenate, due to its structural similarity to phosphate, can replace phosphate during ATP synthesis, thereby disrupting phosphorylation and halting cellular metabolism (NRC, 1999; Villaescusa and Bollinger, 2008; Alpaslan et al. 2010; Basu et al. 2014; Genchi et al. 2022). Recent studies have shown that trivalent methylated arsenic compounds, MMA(III) and DMA(III), may be even more toxic than inorganic arsenic species due to their high potential to cause DNA damage (Vaclavikova et al. 2008). Arsenic toxicity primarily results from its interaction with cellular proteins and DNA regulatory mechanisms. It disrupts cellular energy production, genetic stability, and antioxidant defense. Toxic effects vary depending on dose, compound form, environmental factors, and individual susceptibility (Jones 2007).

Some inorganic arsenic compounds have been used to treat diseases such as malaria, syphilis, leukemia, and psoriasis. However, skin lesions have been observed in patients. After ingestion, arsenic may accumulate in the liver, lungs, kidneys, heart, muscle, and nervous tissues. Within about 2–4 weeks, it begins to accumulate in the nails, hair, and skin (Başkan and Pala, 2009). Once inhaled, arsine (AsH_3) interacts with hemoglobin and is converted into arsenic metabolites, damaging erythrocyte membranes and causing hemolysis. Acute arsine poisoning leads to sudden and severe hemolysis. When inorganic arsenic compounds are ingested orally, they have corrosive effects on the mucosa. After absorption, they increase oxidative stress, disrupt cell signaling pathways, and inhibit certain enzymes. The carcinogenic effects of As^{5+} and As^{3+} have been identified (Sarıhan 2013; Buzoğlu 2019).

The toxic and lethal doses of arsenic vary depending on its form. Oral ingestion of more than 100 mg of inorganic arsenic compounds causes severe toxicity. Ingesting more than 200 mg of water-soluble arsenic trioxide (sodium arsenite) can be fatal. Toxic effects of arsine begin at concentrations above 0.05 ppm in the environment. Concentrations of 25–50 ppm may be fatal within 30 minutes, while 250 ppm causes immediate death (Sarıhan 2013). Humans can ingest up to 300 µg of arsenic daily without showing toxic symptoms. The gastrointestinal absorption rate of inorganic arsenic is very high. About 80% of inhaled arsenic is absorbed. Systemic absorption of arsenic through the skin is not as high as other routes. Arsenic is mainly excreted in urine. It can pass into breast milk and exert toxic effects on infants. It accumulates in hair, nails, and skin (Buzoğlu 2019).

Arsenic poisoning can damage internal organs without showing any external signs, making diagnosis difficult. Before obvious external symptoms appear, elevated levels of arsenic in blood, urine, hair, and nails may indicate arsenic exposure (Hassan 2023).

4.2.1. Acute and Chronic Toxicity of Arsenic

Arsenic can cause both acute and chronic health problems depending on the dose and duration of exposure. Acute toxicity refers to the effects of a toxic substance appearing shortly after exposure. For fish, this period is considered to be 4 days. Chronic toxicity, on the other hand, refers to the effects of a toxic substance appearing over a long period of time (weeks, months, years) (Çetinkaya 2005).

Acute symptoms vary depending on the amount of arsenic exposure, the time of intake, and the patient's age. The intake of 200-300 mg or more of arsenic is sufficient to cause acute arsenic poisoning. Initially, a burning sensation in the mouth and a metallic taste are followed by a short, intense abdominal pain, a feeling of tightness in the throat, bloody-watery diarrhea, loss of appetite, nausea, and vomiting. Cramping in the legs, weak and irregular pulse, pale face, sunken eyes, cold and moist skin, thickening and peeling of the skin on the hands and feet, mouth sores, excessive sweating and hair loss, edema, decreased blood flow, and capillary damage leading to shock can result in rapid severe organ damage, seizures, paralysis, loss of consciousness, coma, and death. Prolonged exposure through drinking water has been associated with skin lesions, vascular diseases, hypertension, increased risk of cancer, late fetal death, and newborn/early infant mortality (He and Charlet 2013; Abdul et al. 2015; Buzoğlu 2019; Bhat et al. 2023; Hassan 2023). In cases of acute arsine gas exposure, there may be no symptoms other than a garlic odor in the first few hours. After a symptom-free period (2-24 hours), clinical findings such as abdominal cramps, blood in the urine and jaundice, chills, shivering, fever, coldness in the extremities, and pain in the head, lower back, and sides may appear (Sarıhan 2013).

Chronic arsenic poisoning results from the intake of slow and non-lethal doses. Gradual onset includes loss of appetite, mild nausea, hair loss, hyperkeratosis on the palms and soles, brittleness and typical lines on the nails (Figure 2), itching, painful swelling, followed by fatigue, diarrhea or constipation, scaling and discoloration that may indicate tumor formation on the skin, black foot disease, muscle weakness, neurological disorders such as paralysis and mental confusion, anemia, colic, and garlic odor on the breath. In addition, perforation of the nasal septum, severe irritation in the larynx and ear canal, anemia, conjunctivitis, tracheitis, and extreme weight loss (cachexia) are among the symptoms. The most affected organs include the skin, bladder, kidneys, liver, and lungs. Its effects are different from acute symptoms and lead to more insidious, long-term damage. In combination with smoking, arsenic exposure can triple the risk of skin cancer by increasing DNA damage (NRC, 1999; Yılmaz and Ekici, 2004; Abdul et al. 2015; Shakoor et al. 2016; Bhat et al. 2023; Hassan 2023). It can disrupt the normal order of DNA, cause overexpression or underexpression of certain genes, and lead to damage or alterations in chromosomes. This may cause uncontrolled cell division, i.e., cancer (Buzoğlu 2019). Some arsenic compounds have also been associated with neurological disorders such as Alzheimer's disease (Basu et al. 2014). Arsenicosis, which results from long-term arsenic exposure, is a health problem that emerges particularly after consuming arsenic-contaminated water above the safe limit (>10 μg/L) for more than 6 months. However, there is no definitive determination of the time required for disease symptoms to appear following the consumption of arsenic-contaminated water (Alpaslan et al. 2010;

Bulam et al. 2010). A study in Japan revealed that after 10 years of arsenic exposure, Bowen's disease developed, after 20 years skin cancer, and after 30 years lung cancer. Skin cancer typically appears after 18 years but can also occur after 30–40 years in some cases (Atabey 2015; Atabey 2019).

Case example: A 47-year-old male resident of Gümüşkaya Village in the Polatlı district of Ankara, who had been farming for 30 years and smoking daily for 37 years, applied to the hospital in 2008 due to the development of numerous skin wounds and spots on his body. It was learned that other individuals in his family and village had similar skin problems. High levels of arsenic were found in the well water used by the village. Upon examination, thick, crusted lesions and areas with suspected cancer were identified on the hands, feet, and face. According to laboratory results, various skin cancers and precancerous lesions were found in the sampled tissues. These findings were associated with the patient's long-term exposure to arsenic. The affected areas were surgically removed and improvement was observed (Bulam et al. 2010).

4.3. Diagnosis and Treatment

The skin system is the most sensitive area to arsenic, and the first signs of arsenicosis appear here (Figure 2). These may include melanosis (dark spots on the skin), keratosis (thickening on the palms and soles), and pigmentation disorders (excessive darkening or lightening). In addition, arsenic accumulation in the nails may cause the formation of white horizontal lines known as Mees' lines. Hair loss (alopecia) is a common symptom of chronic arsenic exposure. Symptoms usually appear 5-10 years after exposure (Caussy and Priest, 2008; Abdul et al., 2015).

Some epidemiological findings showing the effects of arsenic on the skin system include the following: In India (West Bengal), skin thickening and pigment changes were observed in 66% of 156 individuals exposed to 0.05-3.2 mg/L arsenic in drinking water. In Mongolia, hyperkeratosis and discoloration on the trunk were detected in 22% of people exposed to water containing 0.05-1.86 mg/L arsenic. In China (Guizhou), skin lesions were found in 17% of the population exposed to arsenic through water, air, and food due to burning arsenic-rich coal. In Bangladesh, melanosis and keratosis were observed even in areas where arsenic levels were ≤ 10 µg/L, and skin lesions were found to be three times more common in individuals with high urinary arsenic levels. In Sri

Lanka, among 125 people with chronic kidney disease, hyperpigmentation on the palms and soles was observed in 54.4% and 39%, respectively, and keratosis in 23.2% and 17.6% (Abdul et al., 2015).

The brain is one of the main target organs of arsenic toxicity because arsenic can easily cross the blood-brain barrier and spread to all regions of the brain. The highest accumulation is seen in the pituitary gland. It can rapidly cause complications in the nervous system, leading to symmetrical sensorymotor nerve damage. Common symptoms include tingling, pain, and numbness in the soles of the feet. It may cause memory and learning disorders (Caussy and Priest, 2008; Abdul et al., 2015).

Figure 2. Symptoms seen on the skin and nails (Sarıhan 2013; Buzoğlu, 2019)

High arsenic exposure may lead to cardiovascular diseases such as atherosclerosis, hypertension, arrhythmias, myocardial damage, heart failure, and blackfoot disease. Epidemiological studies have demonstrated strong associations between arsenic exposure and heart diseases and mortality rates in countries such as Taiwan, the USA, Bangladesh, and Spain. Prolongation of the QT interval is associated with arsenic exposure and increases the risk of sudden cardiac arrest (Caussy and Priest, 2008; Abdul et al., 2015).

Arsenic affects the hematopoietic system, including bone marrow, spleen, and red blood cells (erythrocytes). It accumulates in the spleen 2–3 times more than in other organs. Arsenic binds to hemoglobin in the blood and causes hemolysis in erythrocytes. As a result, it may lead to blood disorders such as anemia, leukopenia (reduction in white blood cells), thrombocytopenia, and bone marrow suppression (Abdul et al., 2015).

Arsenic can affect the respiratory system through drinking water or occupational environments (such as mining and ore processing). In Bangladesh, individuals exposed to arsenic dust have experienced upper respiratory tract diseases including chronic cough, laryngitis, bronchitis, and rhinitis, as well as deaths (Abdul et al., 2015).

Arsenic is a substance that can disrupt the endocrine system, including the thyroid, pancreas, gonads, and the hypothalamic-pituitary-adrenal axis. It can accumulate in the pancreas, reduce insulin secretion, and impair cell viability. Low-level exposure is associated with hypothyroidism and the development of type 2 diabetes mellitus. It can also lead to fatal complications with symptoms such as nausea, vomiting, diarrhea, and severe fluid loss (Abdul et al., 2015).

Symptoms of liver disease include variceal bleeding, ascites, jaundice, and hepatomegaly. Blood tests may show elevated liver enzymes. Over time, severe liver damage such as hepatomegaly, enzyme elevations, hepatic fibrosis, cirrhosis, and portal fibrosis may develop (Abdul et al., 2015).

By accumulating in the kidneys, arsenic exerts toxic effects on renal tissue. Clinically, signs of kidney damage such as hypourea, elevated serum creatinine, increased blood urea nitrogen, and proteinuria may be observed (Abdul et al., 2015).

Arsenic negatively affects fetal development. Animal studies show that it causes reduced reproduction and development. It can affect reproductive organs, leading to fertility problems and pregnancy complications (fetal loss, preterm birth). Exposure during pregnancy (<50 µg/L) may impair uterine and placental development and reduce birth weight (Abdul et al., 2015).

In cases of arsenic poisoning, treatment must be initiated urgently, as delays may threaten the patient's life. Patients exposed to inorganic arsenic compounds should be undressed and the contact areas should be washed with soap. In poisonings due to ingestion of arsenic compounds, gastric lavage within the first hour is beneficial (Sarıhan, 2013). The most effective drug in acute and subacute arsenic poisoning is generally accepted to be dimercaprol. However, other chelation therapies such as D-penicillamine and sodium dimercaptosulfonate can also be used, including for some skin problems. Skin cancers are treated with standard methods. For skin thickening (hyperkeratosis), topical creams containing salicylic acid can be applied (Buzoğlu, 2019).

5. ARSENIC ANALYSIS METHODS

For groundwater applications, it is highly important to identify individual arsenic species in order to select the appropriate treatment methods and ensure the safety of drinking water. Many scientific studies have been conducted in this field for years, and arsenic analysis methods have become highly advanced. The most sensitive and selective techniques are “coupled systems,” which combine different analytical methods. These include **chromatography** (HPLC), **spectroscopy** (AAS, ICP-MS), optical methods such as **mass spectrometry**, **colorimetric methods** (based on color change), biological sensors (**biosensors**), and **electrochemical** methods (Bhat et al., 2023).

The half-life of arsenic in blood is about 4 days. After oral intake, only a small amount remains in the blood after 10 hours. Therefore, blood tests are generally not useful, though they may help in patients with kidney disease. Since arsenic is mainly excreted through urine, it can be detected in urine within 1-3 days after entering the body. Urine tests are important for distinguishing between harmful and non-harmful forms of arsenic. After fish consumption, arsenic levels in urine may appear elevated, but this does not always indicate poisoning (Buzoğlu, 2019). Chronic accumulation occurs in the lungs, hair, nails, and skin. Since nails grow approximately 0.12 mm per day, arsenic can still be detected in nails even 100 days after exposure. For this reason, arsenic levels in hair and nails are examined in forensic cases. If arsenic levels are above 1 mg/kg in hair and 1.5 mg/kg in nails, the likelihood of poisoning is considered high (Buzoğlu, 2019).

5.1. Analytical Methods

Atomic spectrometry techniques (AAS, AFS, ICP-AES, ICP-MS) are commonly used in the laboratory for total arsenic determination. These methods require expensive and complex instruments as well as trained personnel. If arsenic speciation is desired, these techniques must be combined with separation methods such as HPLC or GC (NRC, 1999; Feldmann, 2008; Bhat et al., 2023).

5.1.1. Spectroscopic Methods

They can detect very low levels of arsenic in water and offer high sensitivity (1 ppb). However, they also have drawbacks such as high cost, limited portability, and time-consuming sample preparation (Bhat et al., 2023):

a). AAS (Atomic Absorption Spectroscopy): AAS is a technique used in combination with a vapor generation unit. In this method, arsenic is converted to its gaseous form (AsH_3), and free arsenic atoms are detected optically. As(V) must be reduced to As(III), using HCl and sodium borohydride ($NaBH_4$). Detection limits as low as 0.26 ppb can be achieved (Feldmann, 2008; Bhat et al., 2023).

b). AFS (Atomic Fluorescence Spectroscopy): Compared to AAS, AFS is more sensitive and has a wider measurement range. It is cost-effective, easy to use, and effective for arsenic speciation. It offers low detection limits from ppb to ppm levels and wide calibration ranges (Bhat et al., 2023).

c). ICP-MS (Inductively Coupled Plasma–Mass Spectrometry): This is the most sensitive method for arsenic detection and allows for the simultaneous determination of multiple elements (Bhat et al., 2023).

d). LIBS (Laser-Induced Breakdown Spectroscopy): This technique creates a plasma by delivering short bursts of laser energy and analyzes the light emitted by the plasma. Water samples are usually pre-concentrated and then absorbed onto surfaces such as zinc oxide for analysis. The detection limit is 83 ppb. Mini-LIBS systems have the potential to work directly with liquid samples (Bhat et al., 2023).

e). XRF (X-Ray Fluorescence Spectroscopy): This method requires pre-concentration on solid surfaces. For example, arsenic is first adsorbed onto alumina particles, which are then centrifuged and analyzed. The detection limit is approximately 0.7 ppb. Some XRF systems are portable (Feldmann, 2008; Bhat et al., 2023).

f). CL (Chemiluminescence): This method is based on the emission of light during a chemical reaction. Arsenic is converted into arsine gas, which reacts with ozone; the light produced is measured. The detection limit is about 1 ppb. Since the system requires a carrier gas tank, its portability is limited (Bhat et al., 2023).

5.1.2. Chromatographic and Combined Methods

These methods allow for the separation of arsenic based on both its ionic and molecular forms. In cases where high sensitivity is required, combining these methods with instruments such as ICP-MS provides the best results. Such combined systems are referred to as hyphenated or coupled techniques (Bhat et al., 2023).

a). IEX (Ion Exchange Chromatography): This method separates molecules based on their electrical charges and is especially important for distinguishing arsenic species. There are two main types: Anion Exchange Chromatography: Negatively charged arsenic species (As^{5+}, MMA, DMA) are retained on positively charged columns. Cation Exchange Chromatography: Positively charged arsenic species (AsB (arsenobetaine), TMAO, TMA) are separated on negatively charged columns (Bhat et al., 2023).

b). IPC (Ion Pair Chromatography): This technique allows for the separation of both ionic and neutral molecules. Neutral columns (such as C18) and ion-pairing reagents are used. The ion pair reagents form temporary bonds with analytes, enabling separation. Many arsenic species can be separated within a short time (Bhat et al., 2023).

c). LC (Reversed-Phase Liquid Chromatography): Arsenolipids found in seafood, which have low water solubility, can be separated using this method. When combined with ICP-MS, its sensitivity increases (Bhat et al., 2023).

d). HILIC (Hydrophilic Interaction Liquid Chromatography): This method can simultaneously separate neutral, cationic, and anionic arsenic compounds that are easily soluble in water (Bhat et al., 2023).

e). SEC (Size Exclusion Chromatography): This technique separates molecules based on size and is used to identify large molecules such as proteins in combination with arsenic species (Bhat et al., 2023).

f). Use of Hyphenated Techniques Combining HPLC with ICP-MS or AAS: Systems such as HPLC-ICP-MS, HPLC-HG-AAS, and HPLC-HG-AES enable detection of arsenic compounds at very low levels. These systems typically operate with reversed-phase chromatography (Bhat et al., 2023).

5.2. Colorimetric Methods

Colorimetry is a technique based on color changes. It can be used in the field. In these methods, detection can be performed with the naked eye, a camera, a UV-Vis spectrometer, or a smartphone. Digital reading improves accuracy. These methods can detect arsenic at levels as low as 1 ppb (Feldmann, 2008; Bhat et al., 2023).

a). Marsh Reaction and Gutzeit Method: Arsine gas (AsH_3) reacts with mercuric bromide ($HgBr_2$) to form a colored stain. The detection limit is 10 ppb. It is inexpensive and widely used.

b). Molybdenum Blue Method: A blue color is formed through the reaction between arsenate and molybdate.

c). Methylene Blue-Based Method: Arsine gas decolorizes methylene blue. Readings can be performed using a digital device.

d). Sulfanilic Acid-NEDA Method: The principle is that As^{3+} reduces sulfanilic acid, and the resulting product reacts with NEDA to produce a magenta (pink-purple) color.

e). Paper-Based Sensor Method: This method is based on the color change of gold or silver nanoparticles deposited on paper upon interaction with arsenic. Its advantages are low cost, portability, and high sensitivity. It can detect As^{3+} with a sensitivity of 1 ppb.

f). Silver Nanoparticle Systems (AgNPs and AgNPr): The basic principle is that silver nanoparticles coated with PEG (polyethylene glycol) interact with As^{3+}, causing aggregation and a color change from yellow to blue. The advantage is that it offers high sensitivity at the 1 ppb level.

5.3. Biological Methods

a). Inorganic arsenic species are measured using light emission and growth inhibition assays of *Vibrio fischeri* bacteria (Villaescusa and Bollinger, 2008).

b). WCB (Whole-Cell Biosensors): These are systems based on genetically modified bacteria such as *E. coli*. They are low-cost and can reach detection limits as low as 1 ppb (Bhat et al., 2023).

c). Aptasensors (Biomolecule-Based Biosensors): Aptasensors use DNA or RNA structures that specifically bind to arsenic species. These systems detect arsenic through fluorescence or color change (Bhat et al., 2023).

5.4. Electrochemical Methods

Nanotechnological advancements have enhanced the sensitivity and selectivity of electrochemical analyses for arsenic detection. Nanomaterials such as carbon nanotubes, metal nanoparticles, graphene, nanorods, and quantum dots modify the electrode surface, improving electron transfer and biocompatibility. As a result, the signal strength and performance of sensors increase. These methods are suitable for the separate detection of arsenic species at low concentrations and can also be integrated into portable systems. While the initial investment cost for sensors is high, they may be cost-effective for multiple tests (Feldmann, 2008; Bhat et al., 2023):

a). Thin-film electrodes based on DWCNTs and graphene offer high transparency and conductivity for As^{5+}, with a detection limit of 0.287 ppb.

b). Polyaniline–nanofiber composite coated with gold nanoparticles works in the 5-400 ppb range for As^{3+}, with a detection limit of 0.5 ppb.

c). Gold nanoparticle-modified porous carbon nitride provides a detection limit of 0.22 ppb.

d). Anodic Stripping Voltammetry (ASV) for As^{3+} and total arsenic, this method was developed by Zhang and Compton, with a detection limit of 0.8 ppb.

6. ARSENIC REMOVAL METHODS

Arsenic pollution is one of the most serious types of environmental pollution caused by human activities. The management of arsenic waste is an important issue both for the environment and human health. Since even very low amounts of arsenic in drinking water have been shown to have serious negative effects on human health, the importance of arsenic removal technologies has been increasing, and the safe disposal of arsenic waste is quite difficult. Therefore, there is a great need to develop effective methods for removing arsenic from drinking water. Arsenic cannot be completely destroyed; it can only be converted into other compounds to become harmless or be stabilized as insoluble compounds. Today, due to the processing of low-quality ores and increased industrialization in developing countries, it is predicted that the amount of arsenic waste will increase even more (Leist et al. 2000; Vaclavikova et al. 2008; Hassan 2023).

In arsenic removal, *physicochemical methods* (adsorption, ion exchange, coagulation and precipitation, diffusion, membrane filtration, permeable reactive barriers, electrokinetic treatment) and *biological methods* (phytoremediation and biological treatment with microorganisms) are the main techniques (Leist et al. 2000; Başkan and Pala, 2009; Basu et al. 2014; Shakoor et al. 2016; Fazi et al. 2016; Hassan 2023).

6.1. Physicochemical Methods

The chemical form of arsenic is important in its treatment. In groundwater, arsenic is generally found as As+3 up to pH 9, and in order to remove it from water, it is converted into the negatively charged arsenate (As+5) form at low pH (pH > 2.2), allowing it to bind electrostatically with positively charged metal hydroxides and be easily removed (Visoottiviseth and Ahmed, 2008; Nicomel et al., 2016; Fazi et al., 2016; Hassan, 2023).

6.1.1. Oxidation

Oxidation does not directly remove arsenic from water; however, by oxidizing arsenite to arsenate, it enables subsequent processes (such as precipitation, adsorption, filtration, etc.) to remove arsenic. Gaseous or liquid chlorine, permanganate, hydrogen peroxide, and ozone are used for arsenic oxidation (Visoottiviseth and Ahmed, 2008).

a). Aeration: This is the simplest oxidation method; however, the oxidation of arsenite with atmospheric oxygen occurs very slowly and may take weeks. Bacteria, strong acidic or basic conditions, copper, activated carbon, or high temperature can accelerate this process. Chemical oxidants (ozone, hydrogen peroxide, chlorine, and permanganate) can oxidize arsenite much faster and under various conditions.

b). Passive Sedimentation: In rural areas, storing water in earthen pots is a form of passive sedimentation. In waters with high iron content and alkalinity, up to 50% arsenic removal can be achieved. However, this method cannot reduce arsenic levels to 10 µg/L, and if initial concentrations are high, it is insufficient. This method has not been successful in Bangladesh.

c). In-situ Oxidation: Water extracted from the aquifer is aerated in a tank and then reinjected into the well. The dissolved oxygen in the water converts arsenite to arsenate and iron to ferric form. Arsenate and ferric hydroxide are then removed by precipitation or adsorption. It can reduce arsenic by up to 50%. It has been applied in Bangladesh. The effectiveness increases when the oxygen content of the reinjected water is raised or more water is reinjected into the aquifer. The advantages are that no chemicals are used and the method is easy to apply, but its effectiveness in arsenic removal is limited.

d). Chemical Oxidation: In this method, arsenite and ferrous iron (Fe+2) are oxidized by directly injecting oxidizing chemicals (such as potassium permanganate) into the subsurface, and the resulting arsenate and iron are removed by precipitation or adsorption. However, this method may have adverse effects on subsurface ecology and water chemistry. Its long-term reliability is questionable. Using this method, Matthess (1981) injected 29 tons of $KMnO_4$ into 17 wells, reducing arsenic concentration from 13600 µg/L to 60 µg/L.

e). Solar Oxidation: This method is based on exposing drinking water in transparent bottles to sunlight. Arsenite is converted to arsenate by UV and oxygen. This natural and inexpensive method is especially suitable for sunny regions. It can reduce arsenic levels by 33%.

6.1.2. Adsorption

Adsorption is a method that enables the separation of compounds in liquid or gas solutions by being retained on solid surfaces. Adsorption occurs through the interaction of gravitational forces with particles on the adsorbent surface. Commonly used adsorbents include activated carbon, activated alumina, iron-based sorbents, zeolites, sand, biomaterials, clay minerals, natural and synthetic oxides, agricultural wastes, and modified activated carbon. This method is over 95% effective in arsenic removal. It does not require chemicals, does not involve complex equipment, and can be easily implemented even in regions with low technological infrastructure. The advantages of the adsorption method include requiring less space, lower chemical use, low cost, and not producing toxic by-products. However, adsorbents become saturated over time, and their performance decreases; therefore, they must be replaced regularly. Additionally, adsorption systems do not automatically detect saturation, so regular monitoring is necessary for quality control (Hassan 2023).

a). Activated Alumina: It has a high surface area (200–300 m^2/g). The best results are obtained at pH between 5.5 and 6.0. At high pH (above 8.2), the surface of alumina becomes negatively charged, and its arsenic retention capacity significantly decreases. Saturated alumina can be regenerated using 4% sodium hydroxide solution; however, the capacity decreases by 30–40% with each regeneration, and complete replacement is required after 3–4 cycles (Başkan and Pala, 2009; Basu et al. 2014; Nicomel et al. 2016).

b). Granular Ferric Hydroxide: Commercially known as AdsorpAs.

Its arsenic binding capacity is 45 g/kg. Compared to activated alumina, AdsorpAs is 5-10 times more effective. It can treat arsenic concentrations down to 10 µg/L. The spent material is non-toxic and does not leach arsenic (Visoottiviseth and Ahmed, 2008; Hassan 2023).

c). Read-F Unit: It is an adsorbent containing ethylene-vinyl alcohol copolymer and hydrated cerium oxide ($CeO_2 \cdot nH_2O$). It does not require oxidation or pH adjustment. It is available in both household and community-scale units. Regeneration is carried out using sodium hydroxide and sodium hypochlorite, followed by rinsing with water and acid neutralization. The spent materials are safely disposed of (Visoottiviseth and Ahmed, 2008).

d). Iron-Coated Sand: In pretreatment, excess iron in water is oxidized and precipitated, then retained with a sand filter. Then, the water is passed through iron-coated sand to remove arsenic. The system can continue arsenic removal through regeneration (Visoottiviseth and Ahmed, 2008).

e). Shapla Filter: A household filter that uses iron-coated brick fragments. 20 kg of filter media can reduce arsenic in 3000 L of water (containing 0.3–0.4 mg/L) to safe levels. It is an inexpensive and practical method. Field trials in rural areas have shown positive results (Visoottiviseth and Ahmed, 2008).

f). Sono Filter: Contains sequential layers of iron filings, sand, charcoal, and brick fragments. It oxidizes As(III) to As(V) and removes it (Visoottiviseth and Ahmed, 2008).

g). Safi Filter: A ceramic candle filter system containing kaolinite and iron oxide. Regeneration is possible but limited to a certain number of cycles (Visoottiviseth and Ahmed, 2008).

h). Activated Carbon: Granular activated carbon allows limited adsorption. Iron-impregnated activated carbons are more successful. It is not economically efficient (Nicomel et al. 2016; Fazi et al. 2016).

i). Local Filters: Filters made using locally available materials such as brick pieces, red soil, clay, iron ore, iron filings, and processed cellulose (Visoottiviseth and Ahmed, 2008).

j). Cartridge Filters: Contain ion-exchange resins or adsorbents. Not suitable for waters with high contaminant loads, as they reach saturation quickly (Visoottiviseth and Ahmed, 2008).

k). Agricultural and Food Industry Wastes: The use of agricultural and food industry wastes as biosorbents is known as biosorption. Arsenic is removed from water through physical and chemical interactions. No nutrients are required; it is easy to use, low-cost, enables arsenic recovery, is reusable, and environmentally friendly. Agricultural wastes such as orange juice pulp, coconut shell, wheat straw, and sugarcane bagasse have shown various capacities to bind arsenic (Shakoor et al. 2016). Biosorbents contain functional groups such as hydroxyl, carboxyl, phenolic, amino, and sulfhydryl, which help remove pollutants. These groups interact with arsenic via ion exchange, complexation, adsorption, and precipitation. For example, -OH and -COOH groups in coconut shell can bind As^{+3}, and polysaccharides in sugarcane bagasse can interact with As^{+5} (Basu et al. 2014; Nicomel et al. 2016; Fazi et al. 2016). Tea waste, wheat straw, and peanut shells removed up to 92% of arsenic from water containing 400 µg/L at low pH (5-6) (Hassan 2023). Cooked mussel shells removed over 95% of arsenic from water (Neisan et al. 2025). Iron oxide-coated fungi, algae, chitosan, plant residues, certain bacteria, and plant roots are also biosorbents (Basu et al. 2014; Hassan 2023).

l). Slag: A by-product formed during steel production containing metal, oxides, ash, and furnace residues. The active sites on its surface chemically react with arsenite or arsenate under basic pH conditions (Hassan 2023).

m). Biochar: A carbon-rich and porous material produced by heating plant or animal waste in an oxygen-free environment at high temperatures (300–800 °C). Suitable pH is required. It can be reused after regeneration (Hassan 2023).

n). Zeolite: It is abundant, inexpensive, and a good alternative for arsenic removal (Hassan 2023).

o). Iron Sulfide Minerals (Pyrite, Pyrrhotite): Iron sulfide minerals (especially pyrite and pyrrhotite) are effective adsorbents for arsenic removal from wastewater. They retain negatively charged arsenic ions on their surface. pH plays a critical role. As^{+5} is rapidly adsorbed on oxidized pyrite surfaces in slightly acidic or neutral environments (pH 3.5-7), while As^{+3} can be easily removed in alkaline conditions (pH 7–9). For example, by adding 5 g/L powdered pyrite at pH 5, the arsenic concentration can be reduced from 10 mg/L to 10 µg/L (Hassan 2023).

p). Laterite and Oxisol Soils: Laterite soil, rich in iron and aluminum compounds and clay, red-colored, can remove more than 80% of arsenic at pH

8.5; Oxisol soil, containing aluminum silicate and quartz, can remove arsenic at pH 5.5 (Hassan 2023).

r). Titanium Dioxide (TiO_2): Due to its low toxicity, chemical stability, and affordability, it is the most commonly used semiconductor photocatalyst in water and wastewater treatment. Under sunlight or UV light, it acts as both a photocatalyst and an adsorbent; in the absence of light, it functions only as an adsorbent. As pH increases, As^{+5} adsorption decreases (Visoottiviseth and Ahmed, 2008; Hassan 2023).

6.1.3. Ion/Anion Exchange

The method is based on the principle that an ion or anion is released from the surface of a sorbent or biosorbent and replaced by an arsenic ion. It uses strongly basic synthetic resins pretreated with chloride, which have high ion exchange capacity. In this method, As^{5+} can be retained by the resins. Therefore, As^{3+} must first be oxidized to As^{5+}. The resins used in the process can be regenerated with sodium chloride (Visoottiviseth and Ahmed, 2008; Shakoor et al. 2016; Nicomel et al. 2016; Hassan 2023).

6.1.4. Coagulation-Flocculation and Precipitation

Coagulation removes negatively charged arsenate ions by neutralizing them with positively charged coagulants ($FeCl_3$, $Al_2(SO_4)_3$). Flocculation allows the agglomeration and settling of coagulated particles. The method works through three main mechanisms in arsenic removal: precipitation, co-precipitation, and adsorption. It generally leads to the formation of insoluble compounds in arsenic-containing water by adding metal salts or lime. The resulting metal hydroxide particles (flocs) adsorb contaminants such as arsenic and then settle. These flocs are later removed by filtration. The process is effective for both arsenate and arsenite at pH < 7.6. Increased temperature, oxygen pressure, and contact time enhance the removal efficiency. More than 90% of arsenic in water can be reduced to below 1 μg/L. However, the disposal of arsenic-laden sludge poses environmental and economic concerns (Visoottiviseth and Ahmed, 2008; Basu et al. 2014; Nicomel et al. 2016; Shakoor et al. 2016).

a). Double Bucket Treatment Unit (BTU): In this method, coagulants such as ferric chloride, aluminum sulfate, and potassium permanganate are added in the upper bucket for sedimentation, and the water is then filtered through sand in the lower bucket to complete the treatment. It is a cost-effective and practical household solution (Visoottiviseth and Ahmed, 2008).

b). Star Filter: Iron coagulant and hypochlorite are mixed in the first bucket, where flocs are formed. In the second bucket, sedimentation and sand filtration are carried out using a specially perforated inner bucket. A cloth filter placed on the sand prevents particle passage. Arsenic can be reduced below 50 µg/L (Visoottiviseth and Ahmed, 2008).

c). Fill-and-Draw Units: A tank is filled with water, to which chemical oxidants and coagulants are added. The water is left overnight for settling. Clear water is then drawn from the top, filtered through sand, and collected from a tap (Visoottiviseth and Ahmed, 2008).

d). Arsenic Removal Units Attached to Hand Pumps: These systems are connected to manually operated hand pumps and are based on aluminum coagulation, sedimentation, and upward flow filtration. Sodium hypochlorite and diluted alum ($Al_2(SO_4)_3$) are added to oxidize and precipitate arsenic (Visoottiviseth and Ahmed, 2008).

e). Iron-Arsenic Removal Units: In waters with high iron content, iron is co-oxidized and precipitated with arsenic. Treatment is carried out through aeration, sedimentation, and filtration. For high removal efficiency, increased contact time between arsenic and iron flocs is necessary (Visoottiviseth and Ahmed, 2008).

f). Lime Treatment: In this method, the addition of quicklime (CaO) or hydrated lime ($Ca(OH)_2$) forms calcium hydroxide flocs that adsorb arsenic. It achieves the highest efficiency at pH 10.6–11.4. However, it requires high amounts of lime (800–1200 mg/L) and produces large volumes of sludge. It is mainly used as a pretreatment step (Güler and Çobanoğlu 1997; Visoottiviseth and Ahmed, 2008).

g). Electrocoagulation: This method provides very high efficiency. Electrical energy is applied to water using sacrificial electrodes. Pollutants either settle or float to the surface and are separated from the water. Iron electrodes provide higher removal. However, electrode corrosion over time, system design, and high energy consumption are drawbacks (Hassan 2023). Using iron electrodes, electrocoagulation can reduce arsenic levels below 10 µg/L (Basu et al. 2014).

6.1.5. Membrane Filtration

Membrane filtration is an advanced water treatment method used for the removal of contaminants. Water passes through membranes due to the pressure difference between their surfaces. The process is not affected by pH or the presence of dissolved ions. However, the presence of colloidal substances and metals such as iron and manganese can cause membrane fouling. Once clogged, membranes cannot be backwashed, and they are also not resistant to oxidizing agents. Therefore, pretreatment is essential (Vaclavikova et al. 2008; Başkan and Pala, 2009; Basu et al. 2014; Nicomel et al. 2016).

There are generally two main types of membrane systems: **low-pressure systems** (microfiltration) and **high-pressure systems** (ultrafiltration, nanofiltration, and reverse osmosis). **Microfiltration** and **ultrafiltration** have larger pore sizes and are only capable of removing arsenic particles in coarse forms. **Micellar-enhanced ultrafiltration** involves the use of surfactants to form micelles that retain arsenic. **Coagulation-assisted microfiltration** works by first transforming arsenic species into precipitable forms via coagulation, followed by their physical separation from water using microfiltration membranes. **Nanofiltration** and **reverse osmosis** are the most effective techniques for arsenic removal, achieving removal efficiencies of 85-99% for arsenate (As(V)) and 61-87% for arsenite (As(III)) (Vaclavikova et al. 2008; Başkan and Pala, 2009; Alpaslan et al. 2010; Basu et al. 2014; Nicomel et al. 2016; Hassan 2023).

6.2. Biological Methods (Bioremediation)

It stands out as a low-cost, environmentally friendly and sustainable solution (Fazi et al. 2016).

6.2.1. Phytoremediation (Plant-Based Arsenic Removal)

Phytoremediation is a method that utilizes plants and bacteria. In particular, *Pteris vittata* (Chinese brake fern) has shown high efficiency in arsenic-enriched soils and can accumulate up to 5.07 g/kg of arsenic in its dry biomass. Although this plant is mostly used for soil remediation, it is also applicable in hydroponic systems. Similarly, *Hydrilla verticillata* achieved 96.4% arsenic removal at an initial concentration of 100 ppb, with a contact time of 5 hours at pH 6. Phytoremediation is a time-consuming process. Plants used in phytoremediation should possess high arsenic accumulation capacity, high biomass yield, a short life cycle, and significant economic value (Visoottiviseth and Ahmed, 2008; Fazi et al. 2016).

6.2.2. Biosorption (Microbial Adsorption)

Biosorption is a heavy metal removal method that utilizes microorganisms or waste biomass. It commonly involves sulfate-reducing bacteria and species such as *Paenibacillus* and *Pseudomonas*. Functional groups on microbial cell walls-such as carboxyl, hydroxyl, sulfate, phosphate, amide, and amino groups-enable the complexation and binding of arsenic. This method is widely applied in wastewater treatment. Its main mechanism involves the oxidation of As^{3+} to As^{5+} by extracellular enzymes, followed by adsorption onto biofilms. Advances in genetic engineering have enabled the development of bacterial strains that overexpress arsenic-binding proteins (e.g., via the *arsR* gene), enhancing biosorption capacity. Some bacteria also use arsenate as a terminal electron acceptor in respiration. Therefore, the biological oxidation of As^{3+} by microorganisms is considered a safer and more economical alternative for arsenic removal (Fazi et al. 2016; Hassan 2023).

6.3. Management of Arsenic Treatment Waste

The safe disposal of the concentrated waste generated from arsenic treatment processes is of critical importance. Arsenic removal systems produce wastes such as arsenic-saturated hydroxides, aluminum oxides, and various filter media. Due to their potentially acidic, basic, saline, and environmentally hazardous nature, these wastes pose significant environmental challenges if not properly managed (Leist et al. 2000; Visoottiviseth and Ahmed, 2008). There are four principal methods for managing arsenic-containing wastes:

a). Marine environment: The marine environment has long been considered a sink for arsenic loads. Marine organisms have the ability to convert inorganic arsenic into less toxic organic forms, thereby reducing its toxicity.

b). Concentration and secure storage: This method is associated with high costs and safety risks. According to regulations in Türkiye, the allowable arsenic content in such waste should range between 0.05-0.2 mg/L.

c). Dilution and dispersion: That involves mixing arsenic-containing waste with other waste materials to lower the concentration of hazardous substances. However, this strategy only ensures compliance with regulatory limits and does not eliminate the environmental risk.

d). Encapsulation (solidification-stabilization): This is considered the most effective method. It involves converting hazardous liquid or solid wastes into less hazardous or inert solid forms, thereby preventing their release into the environment. Arsenic-laden wastes are immobilized using materials such as cement, lime, Fe^{2+}/Fe^{3+} salts, fly ash, silicates, slags, or polymers, and are then safely buried in designated disposal sites. The USEPA considers cement-based solidification as the best available technology for land disposal of toxic wastes (Leist et al. 2000; Alpaslan et al. 2010).

7. REGULATIONS AND STANDARDS

In order for water to be safely used, drinkable, to sustain living organisms, and to irrigate agricultural products, its quality must meet appropriate standards. Countries should take measures for this purpose. These measures should include both the rules for water use and protection. For this purpose, various legislations have been developed in the world and in our country, taking into account drinking, domestic, irrigation, and aquatic life uses. Arsenic, which is among the pollutants that disrupt the natural balance, is important for human and animal health due to its occurrence in free form in nature and its various toxic effects on living organisms (Yılmaz and Ekici 2004; Vaclavikova et al. 2008).

WHO's drinking water quality standards date back to 1958. For arsenic, the initial limit was 200 µg/L, reduced to 50 µg/L in 1963 and temporarily to 10 µg/L in 1993. The EU also temporarily reduced the limit value from 50 µg/L to 10 µg/L in 1998 (He and Charlet 2013; Atabey 2015).

Jordan in 1991, Japan in 1993, South Africa and Syria in 1994, Australia in 1996, Mongolia in 1998, Canada in 1999, Laos in 1999, the USA in 2001, and China in 2006 reduced the maximum allowable arsenic concentration in drinking water to 10 µg/L. Australia later reduced this limit to 7 µg/L. In countries such as Bangladesh, Bolivia, Egypt, Indonesia, the Philippines, Sri Lanka, Saudi Arabia, India, and Chile, where groundwater contains high levels of arsenic, the limit value for arsenic is still applied as 50 µg/L. In Turkey, the arsenic concentration was accepted as 10 µg/L in terms of quality standards with the "*The Production, Packaging, Sale and Inspection of Potable Water Regulation*" in 1997, the "*Water Intended for Human Consumption Regulation*" (Official Gazette No. 2005-25730), and the "*Surface Water Quality Regulation*" (Leist et al. 2000; Yılmaz and Ekici 2004; Başkan and Pala, 2009; Gündüz, 2009; Alpaslan et al. 2010; He and Charlet 2013; Atabey 2015; Genchi et al. 2022; Bhat et al. 2023; Hassan 2023). In the "*Guideline values table for naturally occurring chemicals of health significance in drinking water*" by the World Health Organization (WHO 2022), in the European Union Drinking Water Regulations (SI No. 99 of 2023) (EUDWR 2023), and in various national legislations in our country for both drinking and other uses (WHCR 2005; SWQR 2016; QTWDR 2019), the limit value is defined as 10 µg/L.

8. ARSENIC-CONCENTRATED AREAS

Arsenic, ranked 20th in abundance in the Earth's crust, is widely distributed. In certain geographical regions of the world, it is found in soils and waters at elevated and toxic levels (above 150 µg/L), posing a threat to human health. These countries include India, Bangladesh, China, Taiwan, the United Kingdom, parts of South and Central America, Canada, and New Zealand (Villaescusa and Bollinger, 2008; Atabey, 2019; Buzoğlu, 2019). Globally, more than 100 million people consume drinking water with arsenic concentrations exceeding 50 µg/L (Abdul et al., 2015).

Taiwan was the first place where a serious arsenic-related health problem was observed and documented in 1968. The contamination case in Chile was officially recognized in the 1970s. Problems in countries such as Mexico, India, and Ghana were documented in the 1980s. The largest contamination incident to date has undoubtedly occurred in Bangladesh. In the early 1990s, Bangladeshi patients began crossing the border to seek treatment in hospitals in India; however, official investigations did not commence until 1995. By 1997, it was understood that much of the country was at serious risk (Hassan, 2023).

Fazi et al. (2016) reported that the main sources of arsenic contamination in Italy are geothermal activities and reductive dissolution processes. In this context, arsenic-rich groundwater has been observed in volcanic regions of Latium, Campania, Tuscany, and Sicily. Aquifers formed by waters emerging from wells and springs in these volcanic zones contain arsenic concentrations ranging from 180 to 340 µg/L.

In Turkey, arsenic-contaminated areas are generally associated with geothermal sources and mining activities. These include parts of Central and Western Anatolia, the eastern and western regions of the Black Sea, and some drinking water wells in the city center of Van. Closed basins in Central Anatolia and the inner Aegean region are suitable areas for arsenic formation. Elevated arsenic concentrations have been found in groundwater in geothermal areas in Western and Central Anatolia and in soils and groundwater in regions with mining activities, including the Black Sea belt, Konya Sarayönü, Çanakkale Lapseki, İzmir Bergama, Ödemiş and Beydağ, Kastamonu Bozkurt and Küre, Artvin Murgul, Kütahya Simav, Tavşanlı and Emet, Uşak Eşme, Niğde Ulukışla, Isparta Gölbaşı district, and the Kızılırmak valley in Nevşehir (Gündüz, 2009; Atabey, 2015; Buzoğlu, 2019).

In a study conducted in Simav district of Kütahya, arsenic levels in most groundwater samples (22 out of 28) exceeded the 10 µg/L limit. The maximum value measured was 561 µg/L, with an average of 99 µg/L. In geothermal waters, the average arsenic concentration was found to be 502 µg/L (Gündüz, 2009). Similarly, geothermal sources in the Kütahya region have yielded arsenic concentrations ranging from 80–300 µg/L in Gediz, 70–126 µg/L in Muratdağı (Gediz), 120-125 µg/L in Tavşanlı, 950 µg/L in Yoncalı (Kütahya), 8,900–10,700 µg/L in Emet, 67–106 µg/L in Örencik (Çavdarhisar), 100–900 µg/L in Simav, 662 µg/L in Eynal Hot Spring (Simav), 45.5 µg/L in Yeşil Hot Spring (Emet), 115 µg/L in Dereli Hot Spring, and an average of 376 µg/L in hot waters from the Simav Plain. In Balçova (İzmir), arsenic concentrations of 1419 µg/L in geothermal water, and 63.7 µg/L and 182.4 µg/L in surface waters have been detected, indicating thermal waters as the contamination source (Atabey, 2019). Measurements conducted in İzmir in 2008 found arsenic concentrations of 59 µg/L in Göksu, 32 µg/L in Sarıkız, 10 µg/L in Menemen, and 13 µg/L in Halkapınar, leading to the closure of 29 drinking water wells (Başkan and Pala, 2009). In Antalya's drinking water reservoirs, arsenic levels ranged from 13.01 µg/L in Yeniköy to 8.3-10.04 µg/L in Yeşilbayır (Kır and Ulusoy, 2017). In the Akarçay River (Afyon and Konya), discharge locations of geothermal waters showed concentrations of 2,300–2,400 µg/L (Gençer and Başaran, 2024). In the Bakırçay River and its tributaries (Manisa and İzmir), concentrations ranged from 2-62.3 µg/L (Somay-Atlas and Gemici, 2023).

Several studies have reported arsenic concentrations in the Van Lake

Basin. Faruk Spring in Van's İpekyolu district, considered a healing water by locals, contained 538 µg/L (Yılmaz, 2025). Aygır Lake had an average concentration of 5.06 µg/L, with a maximum of 43.76 µg/L (Çavuş and Şen, 2023). Saline waters outside Van Lake showed concentrations of 52.7 µg/L in Heybeli (Norşin) Lake, 30.2 µg/L and 50.8 µg/L in Ayanis Marshes-1 and -2, respectively, 18.8 µg/L in Amik Marsh, and 87.6 µg/L in Erçek Lake (Atıcı et al., 2021). In central Van, drinking water sources showed 43.1 µg/L in the Veterinary-2 Well, 12.09 µg/L in Kale Spring, 50.08 µg/L in Marangozlar Well, 21.75 µg/L in the Kültür Well, 52.27 µg/L in the Adnan Darendeliler Well, 25.5 µg/L in Bostaniçi Reservoir, 29.05 µg/L in İskele Spring, and 11.22 µg/L in the Yavuz Selim Primary School Fountain (Çavuş et al., 2017). In Erciş district, the Kasımbağı Well showed 12.07 µg/L, the old municipal well in Çelebibağ showed 9.23 µg/L, and the AFAD housing well in Çelebibağ showed 9.63 µg/L (Atıcı et al., 2016). The average concentration in the Çatakdibi River was 4.29 µg/L, and 9.91 µg/L at the Çetintaş site (Aydın, 2019; Aydın and Şen, 2022). On the other hand, Yılmaz and Ekici (2004) reported that arsenic levels in drinking water in the Van region did not pose a risk to human or animal health. Atıcı et al. (2023) and Ömeroğlu et al. (2022) reported 140–263 µg/L of arsenic in Lake Van.

Kaya (2023) reported no arsenic in the Devegeçidi Dam Lake (Diyarbakır); Yelekçi et al. (2012) found levels below 5 µg/L in Kilis city's drinking waters; Topal and Arslan-Topal (2014) measured 2.82–5.2 µg/L in Keban Dam Lake; Fındık and Aras (2023) reported that surface waters in Damsa Dam Lake (Nevşehir, Ürgüp) do not pose an environmental risk; Karadeniz et al. (2024) found levels between 0.27 and 11.87 µg/L in Giresun, with an average of 3.75 µg/L; Yapıcıoğlu et al. (2020) observed no exceedances of the limit value in ten wells in the Harran Plain, with a maximum of 4.12 µg/L; Yazman et al. (2024) reported an average of 0.93 µg/L in Giresun's groundwater; and Kayhan et al. (2006) determined the highest arsenic level in ready-to-eat mussels from Istanbul's fish market to be 0.098 mg/kg.

9. CONCLUSION AND RECOMMENDATIONS

Arsenic is a toxic element that can enter groundwater through both natural and anthropogenic processes, posing serious health risks. Long-term exposure to arsenic, especially through drinking water, can lead to various chronic illnesses, most notably skin and lung cancers. Like the World Health Organization, the European Union, and many other countries, Türkiye has set the maximum safe limit for arsenic concentration in drinking water at 10 µg/L. Exceeding this limit can lead to significant public health problems. The solubility and mobility of arsenic in water depend on environmental conditions such as pH, redox potential, and concentrations of iron and sulfate. Additionally, human activities such as mining, agriculture, and the use of fossil fuels contribute to increasing arsenic contamination.

To address this issue, a variety of arsenic removal technologies have been developed, including oxidation, adsorption, coagulation-flocculation, precipitation, membrane filtration, and bioremediation. However, each method has its advantages and limitations. As a result, research has focused on low-cost, efficient, and sustainable solutions. Promising approaches include electrocoagulation, iron oxide-coated filters, adsorbents derived from agricultural waste, and surfaces coated with biological materials. Nevertheless, advanced technologies often present challenges such as high costs, operational complexity, and the generation of toxic by-products. These factors limit the feasibility of arsenic treatment systems, particularly in rural and low-income areas. Therefore, developing low-cost, energy-efficient, and user-friendly systems remains a critical priority. Moreover, individuals of different ages, genders, and genetic backgrounds may respond differently to arsenic exposure, highlighting the need for more refined epidemiological and toxicological studies. Arsenic intake through food, in addition to drinking water, should also be considered, and comprehensive risk assessment models should be established.

In conclusion, arsenic pollution is a global problem with not only technical but also social and economic dimensions. Effective mitigation requires a combination of advanced technologies and simple, locally appropriate methods. While fields such as nanotechnology and biotechnology offer promising solutions, the systems developed must also be sustainable, cost-effective, and socially acceptable.

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CHAPTER 3

FACTORS AFFECTING HATCHABILITY IN GOOSE BREEDING

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1. INTRODUCTION

Poultry is one of the species utilized in animal production. Among poultry species, geese stand out for their various productivity traits. In the modern poultry industry, chickens are the most widely represented species worldwide, while geese are still raised semi-intensively or intensively. Geese are raised primarily for meat, feathers, fat, and fatty liver. Eggs are not widely consumed but are used primarily for hatching chicks. Goose production is most raised on small family farms (Liu et al., 2021). Geese enter the breeding season at nine to twelve months of age and can lay twenty to eighty or more eggs per season.

Geese are cultivated across a wide geography worldwide, with a high concentration in Asian countries and some Eastern European countries. In addition to chicken, goose and duck meat play a significant role in these countries (Önk and Kırmızıbayrak, 2019; Saatcı et al., 2021). Most of the world's goose population is found in Asia (85%), Europe (9%), and Africa (6%) (Karabulut et al., 2017; Eştürk, 2022). According to FOA data, the top three goose populations in the world in 2023 were China, Mozambique, and Myanmar, respectively, while Turkey ranked 8th (FOA, 2025). In countries where goose farming is intensive, geese are raised for their meat, feathers, liver, and eggs (Tilki and Saatcı, 2016).

While goose farming is found in almost every region in Türkiye, it is most prevalent in the Northeastern Anatolia, Central Anatolia, Western Black Sea, and Aegean regions, and at the provincial level in Kars, Ardahan, Muş, and Elazığ. Additionally, small family farms are found throughout Turkey (TUİK, 2025). Despite our significant global presence in geese, goose breeding has not yet achieved commercial production due to a lack of knowledge about breeding stock, incubation problems, marketing and slaughterhouse issues related to vaccination, care, feeding, housing, and other small-scale operations.

Under normal conditions, hatchability in poultry ranges between 82-85%. Losses of 15-20% occur due to factors such as infertility, egg storage conditions, shell quality of eggs to be incubated, incubator-related errors, feed used to feed breeders, various bacterial and viral diseases, fungal and bacterial contamination, and genetic makeup of the animals, as well as errors in the breeding flock, egg handling, and hatchery management (Türkoğlu et al., 1993; Wilson, 1996; Pas Reform, 1999; Çopur, 2004).

Many factors contribute to hatchability problems in goose breeding. We can group these factors under the main headings of maternal factors, egg factors and incubation factors.

1.1. Maternal Factors

Maternal factors include the breed of the brood geese used for breeding, age, the effect of insemination, their nutrition, stocking density, and infectious agents.

1.1.1. Breed

Geese and ducks are known to have significantly lower reproductive potential than chickens, primarily due to fewer eggs laid during the production cycle and a significantly higher embryonic mortality rate during incubation (Rosinski and Bednarczyk, 1997). The genetic makeup of the animal used in goose farming is a primary factor affecting productivity. The outcome of embryonic development of the offspring during incubation is largely dependent on genetic factors. Hatching efficiency is a heritable trait in poultry. Factors affecting the genetic makeup of poultry include bloodlines, crossbreeding, lethal and semilethal genes, and the age of the animal. It is known that hatchability are reduced in captive breeding without the addition of external breeders. It has been reported that the genotype, origin, and breed of geese have a significant impact on the hatchability and physical characteristics of eggs (Golze 1991; Shalev et al., 1991; Vognivenko and Debrov 1997; Saatcı et al., 2005; Shi et al., 2008; Dermanovic et al., 2008; Salamon, 2020).

Geese can be divided into three groups based on body weight. Based on their production characteristics, they are categorized as meat type (e.g., Embden, Pomeranian), liver type (e.g., Toulouse, Landes), and egg type (e.g., Italian, Czech, Chinese). Reproductive traits such as fertility, hatchability, and egg production of meat and liver type geese are poor to moderate (Salamon, 2020).

1.1.2. Maternal Age

One of the most important factors affecting egg fertility and hatching success is the age of the brood geese used for breeding. It is reported that individuals selected for breeding should be at least seven months old. Eggs from geese younger than seven months old are generally smaller, which can negatively impact hatchability and hatch rates (Önk and Kırmızıbayrak, 2019; Saatcı et al., 2021).

Eggs from young brood geese have thicker shells, which limits gas permeability. Albumin forms a significant barrier to gas exchange between the embryo and the environment during the early incubation period. Brood geese age affects egg weight, as well as the proportions of albumen, yolk, and shell. As age progresses, the yolk ratio increases, while the albumen and shell ratio decrease. Furthermore, albumen quality decreases in older broodstock, leading to increased gas permeability as the shell membrane dries and the albumen liquefies. As broodstock age increases, shell quality deteriorates, permeability increases, and this increases moisture loss during incubation. These negative effects can be partially compensated for by incubation in high humidity. Furthermore, egg weight and shell conductivity have been reported to increase with broodstock age (Britton, 1977; Fletcher et al., 1983; Peebles and Brake, 1987; Meuer and Baumann, 1988).

The use of geese between 2 and 5 years of age is ideal for breeding, both in terms of egg number, egg quality, hatchability, and chick quality. Eggs from geese with high egg production performance also have a high hatchability (Saatcı et al., 2021).

1.1.3. The Effect of Insemination

Insemination in geese is performed in two ways: natural and artificial insemination. Once geese successfully mate naturally, they accept each other and may experience problems if they are forced to switch partners. Because males of heavy goose breeds cannot mate with more than three females, which increases maintenance costs, artificial insemination may be preferred (Tilki and Saatcı, 2016). Geese can lay fertile eggs for 10 days after artificial insemination. Generally, three fertile eggs can be collected after insemination. Sperm can be collected from male geese every three days, and this sperm can be used on 12 female geese. Artificial insemination should be repeated every six days (Saatcı et al., 2021). In a study, it was observed that early embryonic mortality rate was 28.50±12.25%, 86.67±7.07%, mid-embryonic mortality rate was 5.00±0.00%, 5.56±0.00%, late embryonic mortality rate was 34.50±11.94%, 2.22±0.00% in natural mating and offered insemination, respectively (Akinbola and Ewuola, 2024).

1.1.4. Nutrition

In breeding geese, a proper and balanced diet directly affects both egg production and hatchability. Egg quality is critical, particularly for shell thickness, albumen density, and embryo development. Negative effects on these parameters often lead to problems such as embryo mortality during incubation, low hatchability, and decreased offspring quality (Salamon, 2020).

Nutrition in breeding geese directly impacts egg production and hatchability. Inadequate protein, energy, mineral, and vitamin intake leads to embryo mortality, low hatchability, and decreased offspring quality (Salamon, 2020). In particular, deficiencies in vitamins A, D, and E, as well as calcium, phosphorus, zinc, and selenium, negatively impact eggshell quality and embryo development. Inadequate nutrition leads to decreased fertility rates, increased rates of abnormal chicks, and decreased hatchability (Çopur, 2004). Additionally, deficiencies in vitamins A, B_{12}, riboflavin, and pantothenic acid can cause various developmental disorders in the embryo (Salamon, 2020). Micronutrient supplementation has been reported to increase egg numbers but has a limited effect on fertility rates (Janan et al., 2015).

1.1.5. Stocking Density

Stocking density is a significant environmental factor that directly impacts reproductive performance and, consequently, hatchability in geese by affecting both behavioral and physiological stress levels. Excessive density leads to increased social stress, male reluctance to mate, and decreased egg-laying rates (Liu et al., 2021). Furthermore, stress-induced hormonal changes reduce fertilization rates and can negatively impact the shell quality of the resulting eggs (Salamon, 2020).

Under dense flock conditions, hygiene levels often decline; this increases the risk of microbial contamination of eggs, leading to embryo losses and reduced hatching rates (Çopur, 2004; Liu et al., 2021). Inappropriate density conditions impair egg fertilization quality and embryonic development, increasing early embryonic mortality and abnormal hatching during incubation (Janan et al., 2015). For these reasons, population density should be meticulously planned when housing breeding geese; Both animal welfare and breeding and hatching efficiency should be optimized by providing adequate space, feeders, and waterers per bird.

A study with White Sichuan geese showed that high stocking density can reduce antioxidant capacity and suppress growth. The same study concluded that to prevent these negative effects, stocking density should be below 6.15 geese/m² (8.34 kg/m² in live weight) for 14–28-day-old geese and below 4.83 geese/m² (11.4 kg/m² in live weight) for 28–49-day-old geese (Liu et al., 2021).

1.1.6. Infectious Agents

Infectious agents are one of the most important factors affecting hatchability in goose farming. Pathogenic microorganisms such as Escherichia coli, Salmonella spp., Aspergillus fumigatus, and Mycoplasma spp. can cause embryonic mortality, low hatchability, and developmental disorders in chicks. These pathogens are often transmitted to the hatchery environment through contaminated eggshells, inadequate disinfection practices, or infected breeders. Aspergillosis can cause serious respiratory problems and high mortality rates in young geese if the embryo becomes infected during incubation. Bacteria such as Salmonella and E. coli can penetrate the eggshell and infect the embryo, negatively impacting hatchability. Additionally, a study reported that 6 of 121 incubated goose eggs were infected, and 41 experienced embryonic death (Karabulut et al., 2017; Olsen et al., 2017; Anonymous, 2025; Tarazan, 2025). These findings clearly demonstrate the negative effects of infections on the incubation process. Therefore, effective implementation of infection control measures is crucial to increase hatching success.

1.2. Egg Factors

Egg factors include egg quality, fertility (male-female ratio), egg collection frequency, transportation conditions to the hatchery, egg storage conditions, storage duration, and egg cleaning and disinfection.

1.2.1. Egg Quality

The physical characteristics of eggs laid in geese directly affect embryonic development. Broken, misshapen, or contaminated eggs can be consumed as food after collection. Eggs may have some defects in terms of their external appearance and internal structure. Eggs with such defects are not used for incubation. External structural defects in hatching eggs include excessively dirty, cracked, and broken eggs, eggs that are too large or too small, eggs that deviate from their normal shape (round or pointed), and eggs with thin and rough shells. Internal structural defects include double-yolked eggs, yolk loosening, yolk sticking to the shell, and air space displacement. The last three factors are caused by storage errors. Internal structural defects can be eliminated by light inspection (Saatcı et al., 2021). Hatching eggs with such characteristics negatively affect embryo development and reduce hatching rates. The fertility rate in goose eggs ranges from 60% to 90%, and accordingly, hatching rates are similarly between 60% and 90%. In general, hatchability in geese varies between 50% and 90% (Graves, 1985; Puchajda, 1989; Ensminger, 1992; Feltwell, 1992; Önk and Kırmızıbayrak, 2019; Saatcı et al., 2021).

1.2.2. Fertility

In goose farming, using eggs with high fertility is essential for achieving high hatchability. This requires maintaining an optimal male-to-female ratio. Appropriate sex ratios can improve fertilization rates and, consequently, hatchability by increasing mating efficiency (Balko, 1967). The ideal ratio recommended in the literature is 1:3-5 (male:female), which directly affects fertilization success and, consequently, hatchability. When considered alongside the quality criteria of hatching eggs, these factors are critical for sustainable production (Önk and Kırmızıbayrak, 2019; Akın and Çelen, 2020). A ten-year study on Embden and Toulouse geese determined that a ratio of 4 to 6 female geese per male geese optimized fertility and hatchability. Similarly, in White Roman geese, ratios of 1 male to 4 females and 1 male to 6 females have been reported to have positive effects on reproductive performance (Lin et al., 2024). Furthermore, studies in different breeds indicate that sex ratio can affect not only fertility and hatchability rates but also egg production and embryo development. For example, a 1 male to 5 female ratio has been reported to have positive effects on egg production and fertility in Pilgrim geese (Merritt and Gowe, 1956). These findings demonstrate that determining optimal sex ratios in goose farming is critical for increasing hatching success and optimizing production efficiency. The number of females per male goose can vary, but in general, increasing the ratio of females to male goose is preferable as long as it does not affect fertility and hatchability. In breeds such as the Chinese goose, a ratio of 1 male to 5 females should be used, while in heavy breeds, a ratio of 1 male to 3 females should be used. If the male/female ratio in the breeding herd is outside of normal values, fertility problems occur (Gillette D Dale, 1977; Çopur, 2004).

1.2.3. Egg Collection Frequency

One of the most important factors affecting hatching success in goose farming is the frequency of egg collection from nests. Prolonged egg storage in nests, or broody geese resting on eggs, can negatively impact embryo development by exposing them to temperature and humidity fluctuations. This can lead to incubation disruptions such as embryonic mortality, low hatching rates, and decreased chick quality. Furthermore, eggs left uncollected for extended periods become contaminated and more susceptible to microorganism contamination, increasing the risk of infection during incubation. Therefore, regular and frequent egg collection is a critical practice for increasing hatchability and minimizing disruptions (Karabulut et al., 2017; Saatcı et al., 2021).

1.2.4. Conditions for Transporting Eggs to the Hatchery

Environmental factors such as humidity, temperature, and ventilation are crucial when transporting hatching eggs to the hatchery. Suddenly loading eggs that have cooled from storage into a warmer transport vehicle can cause the eggshells to condense due to the temperature difference (Çopur, 2004).

1.2.5. Egg Storage Conditions

Pre-hatching storage conditions for goose eggs directly impact embryo development and hatchability. Storage temperature, in particular, is a critical factor determining embryo viability and hatchability. Studies have shown that storing eggs at 10-15°C and 75-85% relative humidity maintains embryo viability and increases hatchability (American Poultry Association, 2022). Furthermore, during long-term storage (e.g., 24 days), holding eggs at 37.8°C for 5 hours at regular intervals has been shown to support embryo development and significantly increase hatchability (Saatcı et al., 2021). These practices are especially important for maintaining embryo viability during long-term storage. If storage temperatures are not maintained within the appropriate range, embryo development can be negatively affected, and hatching rates can decrease. Therefore, careful attention to the pre-hatching storage temperature of goose eggs is critical for a successful hatching process. Scientific studies in the poultry field have extensively studied the negative effects of egg storage before hatching-on-hatching rates, early embryo morphology, and growth, as well as the effects of physical and environmental conditions such as storage time, temperature, humidity, gas composition, and changes in position during storage (Tazawa et al., 1980). Storage temperatures should range from 10-17 degrees Celsius for storage up to one week and should be lowered to below 10 degrees Celsius for longer storage. Prolonged storage leads to a decrease in the quality of goose eggs (Fasenko et al., 2001; Tilki and İnal, 2003; Salomon, 2020).

Humidity levels play a critical role in embryo development and hatching success during the storage of goose eggs. Failure to store eggs under appropriate humidity conditions can lead to embryo dehydration or microbial contamination due to excessive humidity (Buckland, 1995). Studies indicate that goose egg humidity levels should be adjusted depending on the storage duration. For example, a humidity level of 75% is recommended for eggs stored for 0-3 days, while this level should be increased to 80-88% for eggs stored for 8-10 days. These adjustments support embryo development by minimizing water loss. Furthermore, high humidity levels prevent water loss by increasing the amount of fluid within the embryo, while excessive humidity can create a favorable environment for mold and bacterial growth. This can increase the risk of infection and threaten embryo health (Tilki and İnal, 2004; Salamon 2020; Saatcı et al., 2021). Therefore, careful control of humidity levels during storage is vital for the success of hatching.

1.2.6. Egg Storage Duration

If eggs intended to be stored for more than a few days are not turned daily, this will reduce hatchability. However, excessive turning reduces hatchability (Salamon 2020; Saatcı et al., 2021). Hatching eggs should be transferred to the incubation stage after 3-4 days of storage, and storage should not exceed 7 days. As storage continues, the egg's albumen height, water, and CO_2 content decrease (Çopur, 2004).

1.2.7. Cleaning and Disinfection of Eggs

Not all eggs obtained may be incubated. Some eggs may be dirty. In general, heavily dirty eggs are not used in incubation because cleaning and disinfection are impossible. Lightly dirty eggs can be cleaned and disinfected before use, while clean eggs can be disinfected before use (Saatcı et al., 2021).

Fumigation is performed to reduce embryonic mortality by preventing the negative consequences of microorganisms on the egg's outer surface (Çopur, 2004). Keeping the eggs in the fumigation chamber after fumigation, and inadequate ventilation of the chamber, can have negative consequences on the embryos if some of the fumigation gas remains on the eggs (Saatcı et al., 2021).

1.3. Incubation Factors

Incubation factors include incubation temperature, incubation humidity, egg position and turning, water spraying and cooling the eggs, egg ventilation, and carbon dioxide concentration.

1.3.1. Incubation Temperature

The optimum incubation temperature for poultry is generally between 37-38°C, and deviations from this range can have negative effects on embryonic development, hatchability, and post-hatch performance (Visschedijk, 1991; French, 1997; French, 2009; Kingori, 2011; Bogenfurst, 2017). This temperature range is critical for cell division and maintaining embryo metabolism (Bogenfurst, 2017).

For geese, the recommended temperature range varies depending on the incubation period: 37.8°C for days 1-12, 37.5°C for days 13-23, 37.2°C for days 24-27, and 37-37.2°C during the hatching period. This gradual decrease is intended to offset the embryo's increased metabolic heat production (Bogenfurst, 2017). However, some studies applied a constant temperature throughout incubation, reducing the temperature only by 0.3-0.5°C at hatch (Fasenko et al., 2001; Biesiada-Drzazga et al., 2015). In both methods, the cooling duration and frequency must be adapted according to the embryo's metabolic heat. Temperatures outside the recommended incubation temperature impair embryo development, prolong incubation, and negatively affect embryo viability and hatchability (Salamon, 2020).

1.3.2. Incubation Humidity

Insufficient or excessive humidity is one of the most common causes of mortality during incubation (Amantai et al., 2018). During incubation, gases diffuse through the pores of the eggshell, and this process depends on the functional porosity of the shell and the pressure difference across it (Paganelli, 1980; Mortola, 2009). During incubation, eggs lose weight, primarily to water vapor, and approximately 12–13% weight loss is necessary for the healthy development of the embryo (Meir and Ar, 1991; Meir and Ar, 1996; Bogenfurst, 2017). Excessive water loss can cause the membranes to dry, while insufficient water loss can cause microbial growth and pore blockage, leading to embryo death (Rahn and Ar, 1974; Bogenfurst, 2017). According to Bogenfurst (2017), relative humidity rates should be; 63% on days 1–4, 54% on days 5–12, 56% on days 13–23, 57% on days 24–27, and 77–80% at hatching, respectively (Meir and Ar, 1991; Meir and Ar, 1996; Bogenfurst, 2017). Low humidity causes sticky fuzz, while high humidity causes core closure problems (Bogenfurst, 2017).

1.3.3. Egg Position and Turning

Turning the egg has critical functions, such as preventing the embryo from adhering to the eggshell, preventing temperature elevations in a single region, developing the chorio-allantois sac, ensuring normal yolk-albumin junctions, and the formation of extra-embryonic fluid (Eycleshymer, 1907; Tullett and Deeming, 1987; Deeming, 2002; Çopur, 2004; Kingori, 2011). It also supports embryonic development and albumin utilization (Tullett and Deeming, 1987; Deeming, 1989a; Deeming, 1989b; Deeming, 1991). Studies on domestic poultry indicate that automatic turning of 90° once an hour is sufficient, while turning more than 24 times a day is unnecessary (Eycleshymer, 1907; Chattock, 1925; Olsen and Byerly, 1936; Deeming, 1991; Bogenfurst, 2017).

Goose eggs are placed horizontally, and due to their size, effective turning is even more crucial for embryo development (Buckland and Guy, 2002; Salamon and Kent, 2016; Bogenfurst, 2017; Milojevic, 2018). In traditional systems, manual 180° turning is recommended in addition to automatic turning on certain days (Bogenfurst, 2017). Bogenfurst (2017) suggested placing the eggs at a 45–60° inclination, arguing that automatic turning alone would be sufficient. Milojevic (2018) achieved an 89.77% hatchability success rate with this method. However, Salamon and Kent (2016) showed that this angle is not suitable for large eggs, and that daily manual 180° turning increases yield by 17%. It has also been reported that turning in only one direction can lead to embryo losses (Bogenfurst, 2017).

While no significant difference was found between the direction of manual turning (long or short axis), it was noted that turning along the short axis provides complete rotation, while turning along the long axis is more practical (Salamon and Kent, 2016). In general, results regarding the technique and frequency of turning goose eggs are conflicting, and different application conditions may contribute to these differences.

1.3.4. Egg Water Spraying and Cooling

In natural incubation, the brood hen leaves the nest daily during the second half of incubation. Cooling in artificial incubation mimics this natural behavior and becomes especially important from day 15, when the embryo begins to produce metabolic heat. The temperature of the eggs may be higher than the incubator temperature during this period. Cooling is generally initiated from days 5-8; Initially, it is applied once a day (8-15 minutes) and after day 16, twice a day (20-30 minutes). Neglecting cooling can lead to a decrease in hatchability of up to 20% (Bogenfurst, 2017, Salamon, 2020).

In systems with manual turning, cooling is performed simultaneously with these processes. The application is carried out with fresh air at 20°C, and the egg carts are removed from the incubator. Cooling is continued until the shell temperature of the eggs reaches 29°C. Then, water is sprayed on the eggs at 40°C to compensate for water loss; adding disinfectant to the spray water is recommended to prevent microbial contamination (Bogenfurst, 2017). After cooling, the eggs should be returned to incubation temperature within 30–40 minutes; Otherwise, incubation time may be prolonged, the rate of misplacement may increase, and chick performance may be negatively affected (French, 2009; Bogenfurst, 2017). The temperature, humidity, water spraying, and cooling times required for incubating goose eggs are shown in Table 1 (Saatcı et al., 2021).

Table 1. Temperature, humidity, water spraying, and cooling times required for incubating goose eggs

Day	Dry bulb temperature (°F)	Dry bulb temperature (°C)	Wet Bulb humidity (°F)	Wet Bulb humidity (°C)	Explanation
1	100	37.7	86–88	31.1	No action
2	100	37.7	86–88	31.1	No action
3	100	37.7	86–88	31.1	No action
4	100	37.7	86–88	31.1	No action
5	100	37.7	86–88	29.4	Water spray + cooling 5 minutes
6	99.8	37.6	85	29.4	Water spray + cooling 5 minutes
7	99.8	37.6	85	29.4	Water spray + cooling 5 minutes
8	99.8	37.6	85	29.4	first control and water spraying
9	99.8	37.6	85	29.4	Water spray + cooling 10 minutes
10	99.8	37.6	85	29.4	Water spray + cooling 15 minutes
11	99.8	37.6	85	29.4	Water spray + cooling 20 minutes
12	99.5	37.5	85	29.4	Water spray + cooling 10 minutes
13	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
14	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
15	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
16	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
17	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
18	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
19	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
20	99.5	37.5	85	29.4	Water spray + cooling 30 minutes
21	99.5	37.5	85	29.4	Water spray + cooling 35 minutes
22	99.5	37.5	85	29.4	Water spray + cooling 35 minutes
23	99.5	37.5	85	29.4	Water spray + cooling 35 minutes
24	99.5	37.5	85	29.4	Water spray + cooling 35 minutes
25	99	37.2	85	29.4	Water spray + cooling 35 minutes
26	99	37.2	85	29.4	Water spray + cooling 35 minutes
27	99	37.2	85	29.4	Second check and transfer at the end of the 27^{th} day
28	99	37.2	95	35	No action
29	99	37.2	95	35	No action
30	99	37.2	95	35	No action

1.3.5. Egg Ventilation and Carbon Dioxide Concentration

During the incubation process, ventilation is crucial for ensuring oxygen (O_2) enters the machine and discharging carbon dioxide (CO_2) released from the accumulated eggs. It also helps control the amount of air entering the machine. This prevents incorrect temperature and gas accumulations within the machine (Çopur, 2004). Therefore, ventilation management in goose eggs is critical for optimizing embryo development and hatching success. Ventilation openings should be kept closed during the first days of incubation to quickly reach the desired temperature. Then, they should be gradually opened to 50% by day 12. This opening should be increased to 75-80% between days 12 and 24 and should reach 80-100% by hatching. Similarly, ventilation settings in the incubator should be between 80-100%, depending on CO_2 levels and temperature requirements (Bogenfurst, 2017).

CO_2 is formed during the incubation process because of embryonic metabolism, and at low concentrations (≤0.5%), it supports development by encouraging the embryo to utilize calcium in the eggshell (Tazawa, 1980; Mortola, 2009; Bogenfurst, 2017). While CO_2 concentrations up to 0.5% in the incubator do not negatively affect embryo development, levels above 1.5% can significantly reduce hatching success (Bogenfurst, 2017). For goose eggs, it is recommended that CO_2 levels reach approximately 1% near hatch; this stimulates the embryo to hatch in a low-oxygen (hypoxia) environment. Furthermore, carbonic acid, formed by the reaction of CO_2 with water vapor, dissolves the eggshell, facilitating hatching. However, increased ventilation is essential to ensure the embryo reaches normal oxygen levels after hatching; otherwise, the risk of mortality increases (Bogenfurst, 2017). Because low oxygen levels near hatching can motivate the chick to hatch, a relatively high CO_2 level of 1% is recommended. CO_2 and water vapor react to form carbonic acid, which can dissolve the eggshell and soften it. However, when CO_2 levels exceed 1.5% during incubation, hatching rates can decrease. If normal O_2 levels are not maintained after hatching, chick mortality will occur (Salamon, 2020).

2. CONCLUSIONS AND RECOMMENDATIONS

In goose farming, maintaining a high hatchability each year is crucial for ensuring flock continuity, eliminating breeders with low productivity for any reason, and selecting offspring with high breeding qualities for use in the next production season. Therefore, understanding the factors that cause hatchability problems in goose farming is crucial. Goose breeders need to be informed about this issue and take these factors into consideration for sustainable goose farming.

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CHAPTER 4

WATER FOOTPRINT IN LIVESTOCK FARMING

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Droughts can cause disasters worldwide. The danger of drought arises from exceptionally dry weather, without rain or snow, or from exceptionally low water levels compared to normal conditions. Drought is the result of a water shortage. The risk of drought poses a significant threat to people, animals, plants, and the environment. Drought conditions result from changes in atmospheric conditions. They result from a hydrological imbalance. This can include decreased precipitation over a given period, inadequate or inefficient rainfall, and/or a negative water balance due to increased demand for atmospheric water due to high temperatures or strong winds. Causes or aggravating factors of drought include the absence of snow or glacial melt, low winter precipitation, or rising temperatures. Therefore, water consumption is becoming increasingly important. As users, individuals – whether consumers or producers – must use water more efficiently and not waste it recklessly (GAR, 2021).

Water is a tasteless and odorless substance, necessary for all known life. In small quantities, it is colorless to the naked eye. Regarding the formation of water, wind, waves, ocean currents, and the sun, which activate the water cycle, heat the water in the oceans. The heated water rises with the wind and waves and mixes with the atmosphere. Rising air currents, combined with temperature differences, carry water vapor into the atmosphere. When water vapor and particles encounter cooler clouds, they cause condensation due to the temperature differences. Air currents move clouds around the globe, and cloud particles accumulate, grow, and fall back to Earth from the sky as precipitation in environments with temperature differences. The types of precipitation also vary depending on the magnitude of the temperature difference and its rate. For example, some precipitation returns to Earth in the form of snow, hail, or water. The global water distribution consists of 97.5% oceans and seas and 2.5% freshwater resources. Freshwater resources consist of 31.4% groundwater and 68.3% glaciers. Surface water consists of 87% lakes and 11% wetlands (Anonymous, 2025a). This report aims to provide information on the water footprint, an important parameter regarding the use of scarce freshwater resources.

1. VALUE OF WATER

According to the United Nations World Water Development Report 2021, agriculture accounts for the largest share (69%) of global freshwater consumption. However, water allocation for agricultural purposes is increasingly challenged due to growing competition between sectors and worsening water scarcity. Moreover, water use for food production is often inefficient in many parts of the world. This inefficiency contributes significantly to environmental damage, including depletion of groundwater tables, reduced river flows, loss of wildlife habitats, and pollution. The economic value of water for food production is generally low compared to its value in other sectors. It tends to be particularly low for irrigation of food crops and animal feed, while it can be comparatively high for high-value crops such as vegetables, fruits, and flowers, reaching levels similar to those used in domestic and industrial areas. Assessments of the value of water in food production generally focus only on the direct economic benefits to users, neglecting many other direct and indirect benefits of water, including economic, socio-cultural, or environmental aspects, which are either not quantified or insufficiently considered. These benefits include improved nutrition, adaptation to changing consumption trends, job creation and strengthening the livelihoods of smallholder farmers, as well as contributions to poverty reduction, the revitalization of rural economies, and climate change mitigation and adaptation. The importance of water for food security is substantial, but it is rarely measured and often determined by political imperatives independent of other considerations. Various management strategies can be used to optimize the multiple benefits of water for agricultural production, such as: These include improving water management in rainfed agricultural areas, shifting to sustainable intensification, using irrigation water from natural and unconventional sources, increasing water use efficiency, reducing food demand and therefore water consumption, and improving knowledge and understanding of water use in food production. Improving water security for agricultural production in both rainfed and irrigated systems can contribute directly and indirectly to poverty reduction and reducing gender inequality. Direct impacts include higher yields, lower risk of crop failure and greater crop diversity, higher wages through improved employment opportunities, and stable local food production and prices. Indirect impacts include income and employment multipliers beyond the agricultural sector and reduced migration. Higher and more stable incomes could improve women's education and skills development, thereby encouraging their active participation in decision-making processes. While increasing water productivity can have significant positive effects, potential negative consequences and impacts on poverty reduction, such as land grabbing and increased inequality, must also be considered (Anonymous, 2021).

2.WATER CONSUMPTION IN THE WORLD

The world's water reserves amount to 332.5 million cubic meters, of which more than 96% is salt water. More than 68% of freshwater resources come from glaciers, 30% from groundwater, and 1% from surface waters such as rivers and lakes (a total of 22,300 million cubic meters). Global water consumption has increased tenfold over the last century. By 2025, it will increase by 17% in agriculture, 20% in industry, and 70% for private consumption. As water consumption increases, drinking water resources are also diminishing worldwide due to pollution and industrialization. Today, 20% of the world's population does not have access to safe drinking water. While the amount of water per person was 7,300 m^3 in 2000, it is estimated that it will fall to 4,800 m^3 by 2025. The reasons for this drop in consumption lie in the scarcity and pollution of water resources by precipitation.

According to the UN, 1.4 billion people worldwide lack access to safe drinking water. 470 million people live in water-stressed regions. Each year, 250 million people are infected by water-related epidemics and nearly 10 million people lose their lives. A UN statement on World Water Day, March 22, noted that 4,000 children worldwide die every day (one child every 20 seconds) from contaminated water, and 400 million children lack access to the safe drinking water they need to survive. In developing countries, the amount of water required by a person for daily needs (drinking, cooking, and washing) is 10 liters. The amount of water used for drinking, cooking, and washing is 10 liters. According to a report by the United Nations Environment Programme (UNEP), 2.4 billion people worldwide lack access to safe sanitation. Assuming a 4-liter container is filled with all the water in the world, the amount of water available to living organisms is only one tablespoon. Canada is the most water-rich country in the world, with 92,000 m^3 of water per capita per year, while the United States, the Nordic countries, and Iceland are among the most water-rich countries, with a water potential of over 10,000 m³. The distribution of water resources on Earth is shown in Table 1 (Anonymous, 2025a).

Table 1. Water consumption by some countries per capita (Anonymous, 2025b)

Country	Annual Water Usage (m³, thousand of litres)	Daily Water Useage Per Capita (litres)	Population
Australia	16,130,000,000	1,816	24,329,963
Brazil	63,500,000,000	856	203,218,114
Canada	35,730,000,000	2,722	35,962,234
China	598,100,000,000	1,174	1,396,134,174
Denmark	652,000,000	319	5,591,917
France	29,370,000,000	1,236	65,086,855
Germany	25,330,000,000	856	81,041,582
Greece	9,630,000,000	2,389	11,045,737
India	761,000,000,000	1,677	1,243,481,564
Iran	93,300,000,000	3,638	70,256,316
Iraq	38,550,000,000	2,745	38,469,627
Italy	34,190,000,000	1,546	60,575,316
Japan	81,450,000,000	1,741	128,192,470
Malaysia	11,200,000,000	1,188	25,836,071
Netherlands	8,919,000,000	1,435	17,030,089
Pakistan	183,500,000,000	2,653	189,499,113
Russia	69,500,000,000	1,306	145,778,677
Saudi Arabia	23,350,000,000	2,078	30,782,402
Somalia	3,298,000,000	912	9,909,157
Spain	32,850,000,000	1,926	46,720,188
Türkiye	58,950,000,000	1,978	81,652,088
United States	444,300,000,000	3,732	326,126,497

Although surrounded by water on three sides, Türkiye is considered a water-scarce country. Located in a semi-arid region with extreme temperatures, its water resources consist of natural lakes, rivers, reservoirs, and groundwater. The country has over 120 lakes, of which Lake Van is the largest. There are also over 700 reservoirs, the most important being the Atatürk, Keban, and Karakaya Dams. Türkiye's average rainfall of 643 mm is well below the global average. This volume corresponds to an average of 501 billion cubic meters of water per year. Of this total, approximately 274 billion cubic meters evaporates into the atmosphere, while 69 billion cubic meters infiltrates. Consequently, 158 billion cubic meters of water are transported by rivers of various sizes to closed basins in seas and/or lakes. Of these 69 billion cubic meters of groundwater, approximately 28 billion cubic meters mix with surface water from springs. With 7 billion cubic meters of water from neighboring countries, Türkiye's gross water potential amounts to 193 billion cubic meters. According to the General Directorate of State Hydraulic Structures, the exploitable surface water potential is estimated at 98 billion cubic meters under current technical and economic conditions. Of this total, 95 billion cubic meters come from domestic rivers and 3 billion cubic meters from rivers originating in neighboring countries. Including the 14 billion cubic meters of safe groundwater, Türkiye's net water potential is estimated at 112 billion cubic meters (Anonymous, 2025c).

Table 2. Water use in Türkiye (Anonymous, 2025c)

Year	Irrigation (billion m^3)	Household (billion m^3)	Industry (billion m^3)	*Total (billion m^3)*
1990	22,0	5,1	3,4	30,5
2004	29,6	6,2	4,3	40,1
2008	33,8	5,8	6,0	45,6
2010	38,2	5,8	6,0	49,9
2012	41,6	6,0	8,4	56,0
2014	35,9	5,7	9,1	50,7
2016	43,1	6,2	11,1	60,4
2023	72,0	18,0	22,0	112,0

Türkiye aims to fully exploit its net water potential by 2023. Per capita water supply, calculated at 1,422 cubic meters in 2015, increased to 1,386 cubic meters in 2017 (Anonymous, 2025c).

Compared to European and global countries, Türkiye stands out as one of the most water-scarce countries in terms of per capita water availability. Generally, a country with an annual water potential of more than 5,000 m³ per capita is considered "water-rich." If the Falkenmark indicator for a region is between 1,000 and 1,700 m³ per capita per year, that region is considered to be affected by water stress (Anonymous, 2025c). Per capita water consumption in Türkiye is currently 1,978 m³ (Table 1).

The most important factors influencing water consumption in livestock farming are the species, breed and age of the animal, its physiological condition, housing orientation and quantity, digestive capacity, quality, composition, temperature and access to the water source, feed quantity and dry matter content, wind speed, relative humidity and ambient temperature (Göncü et al., 2008). The daily drinking water requirements of cattle of different age groups are listed in Table 3.

Table 3. Daily drinking water requirements of cattle in different age groups (Göncü et al., 2008)

Animal	Status	Water consumption (L / day)
Holstein Calf	1 month old	5,0-7,5
Holstein Calf	2 months old	5,7-5,7
Holstein Calf	3 months old	8,0-10,6
Holstein Calf	4 months old	11,4-13,3
Holstein Calf	5-9 months old	14-20
Holstein Calf	15-18 months old	23-27
Holstein Heifer	18-24 months old	28-36
Jersey Cow	14 kg/day milk	50-60
Brown, Holstein Cow	14 kg/day milk	55-65
Brown, Holstein Cow	23 kg/day milk	90-100
Brown, Holstein Cow	36 kg/day milk	145-160
Brown, Holstein Cow	45 kg/day milk	182-200
Dry Cow	6-9 months pregnant	35-50
Bull	500 kg body weight	30-45
Bull	600 kg body weight	36-54
Jersey Cow	700 kg body weight	42-63

It is known that animals absorb water through drinking, eating and through metabolic processes in the body.

It has been observed that cows prefer warm water (17–28 °C) to warm or cold water (Andersson, 1987; Lanham et al., 1986; Wilks et al., 1990). Diets high in salt, sodium bicarbonate, or protein increase water consumption, but this is influenced by many factors, such as the water content of the diet.

Water is the most important nutrient for dairy cows. An adequate supply of clean water is generally considered crucial to avoid negative effects on animal health, productivity, and welfare (Beede, 1991; Murphy, 1992; LeJeune et al., 2001). Cows meet their water needs by drinking, ingesting water from feed, and water produced by metabolic oxidation of body tissues. However, the latter method is considered less important. Various studies have shown that, on average, 83% of water intake is through drinking. Dairy cows generally drink large quantities of water. Many environmental factors influence the amount of water dairy cows voluntarily consume. The most important factors are milk production, dry matter intake, diet composition, and ambient temperature (NRC, 2001).

In cattle, water consumption varies between 25 and 160 liters per day (Anonymous, 1998; Peterson, 1999). It represents 15% of live weight (Stallings, 1997).

3. WATER FOOTPRINT

Water plays a crucial role in human health, environmental sustainability, industrial processes, and food security. Global freshwater resources represent only 2.5% of available water. The availability of these limited resources is decreasing due to rapid population growth, global warming, advances in industrial production, and irresponsible consumption practices. In this context, the term "water footprint" is crucial because it promotes efficient use of water resources. The water footprint generally indicates the amount of freshwater required at each stage of a product's life cycle, from production to consumption. Livestock production accounts for a significant portion (about one-third) of the global agricultural water footprint. Research has shown that the water footprint of meat production exceeds that of milk and egg production, with beef accounting for the largest share of meat production. The fact that agriculture accounts for 89% of Türkiye's water footprint underscores the importance of efficient water management in animal feed production. As Turkish animal feed production is expected to increase with global population growth, this trend is likely to increase pressure on existing freshwater resources. Therefore, it is important to understand water requirements at all stages of animal feed production to ensure efficient management and optimal water use. Other strategies to reduce the water footprint in animal production include using low-water-footprint feeds, promoting pastoral management, reducing food waste, and raising awareness among all stakeholders in the production chain (Kaya and Mazlum, 2025).

The concept of water footprinting was first introduced by Arjen Hoekstra in 2002 as a measure of water consumption. Collaboration between leading global organizations in this field led to the creation of the Water Footprint Network in 2008. This network aims to coordinate initiatives to develop and disseminate knowledge on water footprinting concepts, methods, and tools. Interest in water footprinting is based on the recognition that impacts on freshwater systems are ultimately linked to human consumption. By considering the entire production process and supply chain, issues such as water scarcity and pollution can be better understood and addressed (Dhlamini et al., 2013).

The water footprint consists of three components: the green, blue, and gray water footprints, which represent water consumption and quality. The green water footprint describes the total amount of rainwater needed to produce a good. This is precipitation stored in the soil and absorbed by plants through transpiration and evaporation. In addition, the green water footprint refers to terrestrial precipitation that does not mix with groundwater but is stored in the soil or remains there. The blue water footprint describes the amount of water withdrawn from surface or groundwater resources, evaporates, is added to a product, is withdrawn from one body of water and returned to another, or is returned to the same body of water at a later time. The gray water footprint describes the amount of freshwater needed to absorb pollutants to meet certain water quality criteria. The gray water footprint includes point source pollution, which is discharged directly from a pipeline into a freshwater source or indirectly through surface runoff or leakage from soil, impervious surfaces, or other diffuse sources. The water footprint caused by production in Türkiye is 17% (Anonymous, 2025d).

Globally, pasture is the largest contributor to the water footprint of animal feed (38% of the total water footprint), followed by corn (17%), forage crops (8%), soybean meal (7%), wheat (6%), barley (6%), and oats (3%). In general, concentrates have relatively higher blue and gray water footprints, while byproducts, residues, and forage crops have relatively lower water footprints. Industrial systems use large quantities of concentrates, which generally have higher blue and gray water footprints than pasture and forage crops. As dietary habits shift toward increased consumption of animal products, the pressure to increase production is increasing. This also drives a shift to industrial systems capable of achieving higher unit yields. With the increase in production and the shift to more industrial systems, more concentrates are being used, increasing the water footprint of the entire livestock sector. This is particularly evident in the increase in the blue and gray water footprint per unit of product. Considering the water requirements of animal products; 196 liters for 1 egg, 79% green, 7% blue, 13% gray; 1 glass (250 ml) of milk: 255 liters, 85% green, 8% blue, 7% gray; 1 kg of chicken: 4,325 liters, 82% green, 7% blue, 11% gray; 1 kg of red meat (small ruminants): 10,412 liters, 94% green, 5% blue, 1% gray; 1 kg of red meat (beef): 15,415 liters, 94% green, 4% blue, 3% gray are used. The water footprint of production in Türkiye is approximately 139.6 billion m3/year. 64% of the water footprint of production in Türkiye is the green water footprint, 19% is the blue water footprint, and 17% is the gray water footprint (Anonymous, 2025e).

While the water footprint of concentrate feed was 1,048 m³/tonne, that of roughage was 203 m³/tonne (Mekonnen and Hoeksrta, 2012). They concluded that the water footprint (blue and gray) of animal products is the highest in industrial systems, with the exception of chicken products. The water footprint of each animal product exceeds that of plant products with similar nutritional value. Ultimately, 29% of the total water footprint of global agriculture is attributable to the production of animal products, with livestock accounting for one-third of the global water footprint of animal production.

Table 4. Global water footprint of animal species (Mekonnen ve Hoeksrta, 2012)

Animal species	Average water footprint of the animal throughout its life (m^3/animal)	Annual average water footprint of animal (m^3/animal)	Share in total water footprint (%)
Chicken	6	26	11
Sheep	141	68	3
Beef Cattle	1889	630	33
Dairy Cattle	20558	2056	19
Horse	19189	1599	7

According to Table 4, dairy cows have the largest ecological footprint, although milk production must be taken into account. Whether milk or meat, the water contained in milk is transferred to humans through consumption. Milk contains approximately 88% water, meat less. Water does not remain within an organism, but passes from one organism to another, whether plant or animal.

According to Table 5 below, animal products are classified according to their average water footprint as follows: milk < eggs < chicken meat < butter < mutton/goat meat < beef.

Table 5. Global water footprint averages in animal producs (Mekonnen and Hoeksrta, 2010)

Water footprint per unit live weight (L/kg)					Nutrient content			Water footprint per unit of nutritional value		
Products	Water sources				Calorie	Protein	Fat	Calorie	Protein	Fat
	Green	Blue	Grey	Total	kcal/kg	g/kg	g/kg	L/kcal	L/g	L/g
Milk	863	86	72	1021	560	33	31	1,82	31	33
Eggs	2592	244	429	3265	1425	111	100	2,29	29	33
Chicken meat	3545	313	468	4325	1440	127	100	3	34	43
Butter	4695	465	393	5553	7692	0	872	0,72	0	6,4
Sheep/goat meat	8253	457	53	8763	2059	139	163	4,25	63	54
Beef	14414	550	451	15415	1513	138	101	10,19	112	153

The first studies on daily water intake were conducted by Winchester and Morris in 1956. In this study, they used data on temperature, body weight, and dry matter intake. Later, Hicks et al. (1988) developed an equation that considered temperature, dry matter intake, precipitation, and dietary salt content. While temperature and dry matter intake showed a positive correlation, precipitation and dietary salt content showed a negative correlation.

The blue, green, and gray water footprints, as well as the total water footprint of beef production in Türkiye, are higher than those of global beef production. The average water footprint of beef production and meat processing in Türkiye is higher than the global average (Demir, 2023). The water footprint of beef, expressed in terms of live weight, carcass weight, and red meat, is shown in Table 6 below.

Table 6. Water footprint values (L/kg) of cattle raised in different production systems in Türkiye according to live weight, carcass and red meat yield (FAO, 2016)

Product	Water source	Grazing	Mixed	Industrial	Water footprint L/kg Türkiye	Water footprint L/kg World
Live weight	Blue	316	330	650	369	256
	Green	10596	7318	5690	8383	7002
	Grey	268	257	642	313	219
	Total	11180	7905	6982	9065	7477
Carcass	Blue	537	560	1088	627	389
	Green	17502	12088	9399	13910	10234
	Grey	443	425	1061	519	320
	Total	18482	13073	11548	15056	10943
Red meat	Blue	759	792	1536	886	550
	Green	24650	17025	13239	19591	14414
	Grey	623	599	1494	731	451
	Total	26032	18416	16269	21209	15415

Winchester and Morris (1956) found that dairy cows consumed 1.40 liters of water per kg of feed at 4.4°C, 1.51 liters at 10°C, 1.74 liters at 15.6°C, 2.04 liters at 21.1°C, 2.35 liters at 26.7°C, and 3.33 liters at 32.2°C. Melin et al. (2005) found that dairy cows in milk production consumed an average of 84 liters of water per day. Cardot et al. (2008) determined a daily water consumption of 82.0 liters.

In Hoffman and Self's (1973) study, cattle consumed 31.2 liters of water in summer and 19.0 liters in winter. Arias and Mader (2011) reported that cattle's water consumption was 87.3% higher in summer than in winter. Daily water consumption plays an important role in regulating cattle's body temperature in summer. Hoffman and Self (1973) found that cattle in shade consumed 30.1 liters of water, while cattle without access to shade consumed 32.6 liters.

Ittner et al. (1951) found that the average daily water consumption was 58.14 L at a water temperature of 18 °C and 62.87 L at a water temperature of

31.2 °C. Parker et al. (2000) determined a daily water consumption of 35.6 L for cattle.

Loneragan et al. (2001) observed a positive relationship between wind speed and water consumption. However, wind speed only explained 0.5% of the variation in water consumption. Sexson et al. (2012) found a positive effect between water consumption and mean atmospheric pressure, and a negative effect on water consumption at high and low atmospheric pressure.

Meyer et al. (2004) studied the daily water consumption of dairy cows. They conducted two experiments, each with 60 Holstein cows. Daily water consumption per cow ranged from 14 to 171 kg, with an average of 82 kg. Using multiple regression analysis, they obtained the following equation: Water consumption (kg/day) = -26.12 + 1.516*average ambient temperature (°C) + 1.299*milk production (kg/day) + 0.058*body weight (kg) + 0.406*sodium intake (g/day). The coefficient of multiple determination (r^2) was 0.60.

Meyer et al. (2006) found that daily water intake in Holstein bulls was 17.8 L. Cardot et al. (2008) found a positive correlation between dry matter intake and water intake.

Relative humidity, also, affects water consumption in cattle. A slight increase in water consumption was observed when relative humidity was below 50%. However, for every 10% increase in relative humidity, water consumption decreased by one liter per animal per day when relative humidity was above 50%. At temperatures below 24°C, humidity has little effect on water consumption. In contrast, at higher temperatures and humidity, water consumption decreases (Ragsdale et al., 1953). Relative humidity has no effect in summer, while water consumption decreases with increasing humidity in winter (Arias and Mader, 2011).

Arias and Mader (2011) developed three different equations based on daily water consumption: the summer estimate, the winter estimate, and the general estimate. In the summer and general models, dry mass consumption, solar radiation, and minimum temperature determine daily water consumption, while in the winter model, dry mass consumption, solar radiation, maximum temperature, wind speed, relative humidity, and precipitation explain daily water consumption. While the summer and winter models explain 23% of daily water consumption, the general model explains 64%. The factor that most influences the summer model is solar radiation.

Table 7. Influence of breed composition on water intake (WI) of growing beef cattle (Brew et al.,2011).

Breed	Gross WI, L/head/d	WI/kg Metabolic BW, L/head/d
Charolais X Angus	42.8	0.58
Angus X Brangus	30.8	0.42
Brangus	30.8	0.32
Charolais X Brangus	29.7	0.38
Brangus X Romosinuano	24.1	0.28
Charolais X Romosinuano	20.7	0.32

Brew et al. (2011) conducted a study to assess water intake in adolescent cattle aged 7–9 months and to investigate the influence of breed, sex, dry matter intake, and body weight gain on water intake. Adolescent bulls, steers, and heifers were housed in an open-housing system for 13 weeks. Feed and water intake were measured individually in groups of 16–18 animals. Mean water intake was 29.98 L ± 8.56 L per animal per day. Brahman and Romosinuano cattle consumed less water than British and Continental breeds of the same metabolic body weight (Table 7). No differences in water intake per kg metabolic body weight were observed between bulls, steers, and heifers. Throughout the study, average daily temperature remained within the thermal neutral range and had no effect on water consumption. A positive correlation was observed between water consumption and feed intake, as well as body weight gain. No correlation was found between water consumption and the weight gain/feed ratio.

The factors influencing water consumption, although different between summer and winter, were determined as ambient temperature, relative humidity, low, average and high atmospheric pressure, wind speed, body weight, metabolic weight and maximum temperature of the previous day. Body weight and metabolic weight were determined as linear variables, while average humidity, maximum temperature, relative humidity and maximum and minimum temperatures of the previous day were determined as quadratic variables (Arias and Mader, 2011; Sexson et al., 2012).

Daily water consumption is influenced by dry matter intake and body weight. Animals tend to consume a lot of dry matter and little water during the winter months (Arias and Mader, 2011). Hick et al. (1988) found a positive correlation between dry matter intake and daily water intake.

Sexson et al. (2012) observed that in cattle weighing less than 500 kg, water consumption increased by 22 to 38 liters per animal with increasing body weight, while in animals weighing more than 500 kg, water consumption decreased with increasing body weight. This is explained by lower protein and water retention with increasing body fat percentage. They observed that water consumption per animal increased by approximately 13 liters when the daily ambient temperature increased from 25 °C to 45 °C. They observed that water consumption decreased at temperatures below 15 °C and found that every 1 degree decrease reduced daily water consumption by 0.5 liters.

Since then, the water footprint (WF) has become a key indicator of the sustainability of agricultural water use. To better understand the water requirements of beef and sheep production in grazing systems, it is essential to assess the WF of each farm. Therefore, Murphy et al. (2018) conducted a study to identify the main factors contributing to on-farm freshwater consumption, expressed as volumetric WF, and to analyze the impacts of producing 1 kg of beef and 1 kg of sheep on different grazing farms for two consecutive years, 2014 and 2015. They found that WF accounts for green water, which comes from soil moisture uptake through evapotranspiration, and blue water, which comes from groundwater and surface water use. They also calculated the impact of freshwater consumption on the overall water stress associated with beef and sheep production in Ireland. They found that the average WF for beef farms was 8,391 liters of water per kg carcass weight (CW), of which 8,222 liters/kg CW was green water and 169 liters/kg CW was blue water; 88% of the WF was water used for pasture production (including silage and grass), 10% for concentrate production, and 1% for on-farm water use. The average stress-weighted WF for beef was 91 liters of water equivalent per kg CW, meaning that producing each kg of beef in Ireland is equivalent to the average global citizen's freshwater consumption of 91 liters. For sheep farms, the average WF was 7,672 liters/kg CW, of which 7,635 liters/kg CW was green water and 37 liters/kg CW was blue water; Pasture production contributed 87% of WF, concentrate production 12%, and on-farm water use 1%. The average stress-weighted water requirement of sheep was 2 liters of water equivalent per kg of body weight. The study also assessed the sustainability of recent intensification efforts in Ireland, showing that increased productivity was due to increased green water use and higher grass yields per hectare on both cattle and sheep farms.

4. CONCLUSION

Decision-makers are confronted with climate change, economic crises, wars, and population growth. This situation requires revisiting traditional management approaches and identifying the flaws and deep uncertainties that contribute to delays. Three research questions on the future of water resources management are addressed: redefining multidisciplinary science, analyzing innovative collaborations, and solving complex problems; creating a science-based policymaking culture and enabling effective communication and sustained engagement to accelerate policy responses so that decision-makers understand and adopt research and technological advances; and making decisions in a context of profound uncertainty. In this context, water resources management must overcome existing infrastructure problems, water scarcity, degrading water quality, extreme natural events, human, economic, and institutional mismanagement, and the impacts of climate change by supporting hydropower and agricultural production.

It is reported that the largest share of the global water footprint (≥ 70%) is attributable to agricultural activities, with one-third of this being attributable to livestock production.

In animal production, it has been found that beef production requires more water than milk and egg production. It is expected that as the world's population increases, animal-based food production will also increase.

Developing and growing societies appear to face significant challenges in accessing clean, fresh water. Water scarcity, pollution, and insensitivity in its use could lead to social and economic problems in the future. Consequently, water wars are likely. To avoid this, rational water management is essential.

Plants can hardly cover all of a person's protein needs. Therefore, it is necessary to cover part of this through meat, milk, eggs, etc., from animal-based foods. Water consumption in meat production can be optimized by producing water-efficient feed. Therefore, knowledge of the water consumption of the raw materials used is essential for water management and efficiency. In animal feed, water consumption can be reduced by using water-efficient feed, promoting pasture farming, systematic grazing, building modern stables, shelters, and slaughterhouses, raising awareness among producers, and, above all, preventing food waste. Since water scarcity, pollution, and excessive water consumption can lead to ecological and social problems, we must ensure more economical use of freshwater resources.

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CHAPTER 5

SEAFOOD CONSUMPTION FROM A GLOBAL AND LOCAL PERSPECTIVE: HABITS, FACTORS, AND SUSTAINABILITY

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INTRODUCTION

Aquatic products are not only a basic food source for billions of people worldwide, but also a significant economic activity and an integral part of cultural heritage. Their richness in protein, essential fatty acids (omega-3), vitamins (D and B12), and minerals (iodine, selenium, and zinc) makes them an essential component of healthy diets. This nutritional richness contributes to a wide range of human health, from cardiovascular health and brain development to strengthening the immune system and preventing chronic diseases (FAO, 2022). Global population growth, urbanization, and rising income levels are constantly increasing the demand for aquatic products. Increasing affluence, especially in developing countries, is leading to a shift towards more diverse and high-quality food sources, which in turn encourages the consumption of aquatic products. Increasing awareness of healthy eating is also a key factor supporting this demand. Consumers are turning to lighter, more digestible, and more nutritious aquatic products as an alternative to red meat (Choudhury, et al., 2022). The "State of World Fisheries and Aquaculture" (SOFIA) reports published by the Food and Agriculture Organization of the United Nations (FAO) clearly demonstrate this global trend. Annual per capita consumption of aquaculture products, which averaged 9 kg in the 1960s, reached 20.2 kg by 2020 (FAO, 2022). Revolutionary developments in the aquaculture sector have played a key role in this increase. Aquaculture reduces pressure on natural fish stocks while providing a stable and sustainable production model, making significant contributions to meeting global demand. In 2020, approximately 49% of total aquaculture production came from aquaculture, highlighting the central role of aquaculture in future food security strategies (FAO, 2022).

Aquaculture consumption habits vary greatly across the world due to geographical, cultural, economic, and even religious factors. These differences shape both production and consumption patterns:

<u>Asia:</u> The Asian continent, which accounts for approximately 70% of global aquaculture production and consumption, is the undisputed leader in this area. China, in particular, stands out with both its massive production capacity and high per capita consumption rates. Japan, South Korea, and Southeast Asian countries, where fish and seafood have traditionally been a significant part of the diet, also have high consumption levels. In these regions, aquaculture

is not only a source of nutrition but also a key element of cultural identity and culinary traditions (Kobayashi et al., 2015).

Europe: In Europe, aquaculture consumption is particularly high in Northern European countries (Norway, Iceland) and Mediterranean countries (Spain, Portugal). Fishing has a long tradition in these countries, and easy access to fresh seafood and traditional culinary habits support consumption. Average per capita consumption is around 22 kg/year (Richter, et al., 2017).

North America: While aquaculture consumption in North America is lower than in Asia and Europe, it is on the rise due to increasing awareness of healthy living and sustainability. Consumption rates are particularly high in coastal areas and where local fishing cultures are strong (Shamshak, et al., 2019).

Africa and the Middle East: Seafood consumption in these regions is generally lower due to low income levels, supply chain constraints, and difficulties accessing fresh produce. However, in some coastal areas, local fisheries are an important source of income and food (Bonanomi, et al., 2017).

Global seafood demand is expanding not only in quantity but also in product variety and presentation. Consumers are turning to prepared and processed products such as canned tuna and frozen seafood, in addition to traditional fresh fish. The popularity of cuisines from different cultures, such as sushi and ceviche, is diversifying seafood consumption among younger generations. Furthermore, demand for organic, low-mercury, and traceable certified products is increasing, driven by health and environmental concerns. Plant-based seafood alternatives (e.g., algae-based fish products) are also gaining market share (Troell, et al. 2014).

Increasing aquaculture consumption is putting significant pressure on natural fish stocks. According to FAO data, approximately 35% of the world's fish stocks are overfished (FAO, 2022). This poses significant threats to the health of marine ecosystems and biodiversity. Therefore, expanding sustainable fishing practices and reducing the environmental impacts of aquaculture are crucial. Sustainable fishing encompasses management approaches that aim to protect the renewability of fish stocks and the health of marine ecosystems. Certification systems such as the Marine Stewardship Council (MSC) are raising awareness in this area by helping consumers choose products from sustainable sources. In aquaculture, innovative production techniques such as closed-loop systems and integrated multitrophic aquaculture (IMTA) aim to reduce the environmental footprint and ensure more efficient production (Troell, et al. 2014). With the global population expected to reach 9.7 billion by 2050, the strategic role of aquaculture in meeting protein needs will become even more evident. FAO projections predict that global aquaculture production could exceed 200 million tons by 2030, with aquaculture remaining the primary driver of this growth. Technological advancements will enable significant advances in traceability, quality control, and food safety (FAO, 2022, Gonzalez-Fernandez and Ugarte-Urra, 2021). However, it is crucial to remember that this growth will not be sustainable unless supported by environmentally responsible policies and conscious consumer behavior. Seafood consumption is a critical issue for individual health and global food security. While consumption trends are diversifying and shifting toward health-focused preferences, environmental sustainability is the most vulnerable aspect of this growth. International collaborations, ethical production, conscious consumption, and environmentally friendly policies are key to sustainable development in this area. This study comprehensively examines trends, regional differences, determinants, and sustainability approaches in seafood consumption at global and local scales. Furthermore, consumption dynamics and sustainable practices specific to Turkey are also discussed in detail.

2. Global Consumption Trends and Geographic Distribution

Seafood consumption has increased significantly worldwide in recent years. The primary drivers of this increase include global population growth, rising income levels in developing countries, and a growing awareness of healthy eating in developed countries (Choudhury et al., 2022). Asia, in particular, leads in seafood consumption. China accounted for approximately 35% of global seafood consumption in 2011, with per capita consumption reaching 38 kg (Kobayashi et al., 2015, Khora, 2020). This reflects the central role of seafood in Chinese cuisine and culture. However, seafood consumption in China is projected to surpass domestic production by 2030, increasing the importance of imports (Crona et al., 2020). Across Asia, seafood remains a staple in many diets. In Japan, annual per capita consumption is approximately 50 kg, with a large portion of the population consuming seafood several times a week (Almeida et al., 2015; FAO (Food and Agriculture Organization).

(2020)). Southeast Asian countries also have high consumption rates; for example, in Malaysia, average per capita seafood consumption in coastal and rural areas has been reported as 44.53 kg/year (Von Goh et al., 2021). The rich marine biodiversity and traditional fishing methods in these regions reinforce the place of seafood in the daily diet. In Europe, average per capita seafood consumption is around 22 kg/year (Richter et al., 2017). Mediterranean countries such as Portugal and Spain have high consumption rates, exceeding 30 kg per capita, thanks to their traditional diets heavy in fish and shellfish (Almeida et al., 2015, Pita et al., 2022). In contrast, in some Western countries, such as Australia, per capita consumption remains around 15 kg per year, despite positive attitudes toward seafood, due to barriers such as taste preferences and ease of preparation (Birch et al., 2012; Birch and Lawley, 2013). This clearly highlights regional differences in consumer habits and market dynamics. In North America, particularly in the United States, per capita seafood consumption is approximately 20 kg, with regional variations observed (Shamshak et al., 2019). For example, in regions with strong local fishing practices, such as Hawaii, recreational fishermen report high consumption rates, averaging around 45 kg per year (Pita et al., 2022). These data suggest that seafood consumption is closely linked not only to global trends but also to local cultural practices and accessibility.

3. Factors Influencing Consumption Habits

Seafood consumption habits are shaped by a complex interaction of cultural, economic, environmental, and social factors. Religious and cultural traditions, parental attitudes, and regional culinary practices significantly influence individuals' perceptions and consumption preferences toward seafood (Govzman et al., 2020). For example, in Portugal, cod, despite not being native to the country's waters, has become a fundamental part of the national cuisine due to historical, political, religious, and traditional reasons (Almeida et al., 2015). In China, the consumption of luxury seafood is linked to historical trends, traditional Chinese medicine, and social stratification, demonstrating the interplay of cultural and economic factors on consumption (Fabinyi, 2011). Different seafood processing methods and dietary habits in Asian countries contribute to differences in the sensitivity profiles of individuals with seafood allergies compared to Western countries (Khora, 2020). Positive attitudes toward seafood are associated with more regular consumption, while familiarity and lack of habit can lead to lower consumption levels (Birch and Lawley, 2013). Family factors, attitudes toward sustainable seafood, and ethical consumption influence the formation of consumption habits, particularly in children aged 5–11 (Musarskaya et al., 2017). This highlights the impact of dietary habits acquired early in life on consumption patterns in adulthood. In the United States, seafood consumption patterns vary in terms of resource use, expenditure, and regional differences (Love et al., 2020). In Norway, frequent consumption of seafood, especially for lunch, is a common practice (Wiech et al., 2021). Consequently, seafood consumption habits depend on numerous factors, including cultural traditions, historical trends, social status, and regional culinary practices. Understanding these interactions is critical to promoting sustainable seafood consumption and addressing differences in dietary habits across cultures. As global demand for seafood continues to increase, sustainable consumption practices become essential to ensure the health of marine ecosystems and the availability of seafood for future generations (Richter, et al., 2017; Bonanomi, et al., 2017).

4. Global Inequalities and Market Dynamics

Significant inequalities in seafood consumption are observed globally. For example, countries like Portugal, Japan, and the Maldives lead in annual per capita consumption, while consumption rates are significantly lower in landlocked or developing countries like Afghanistan, Uganda, and Niger. These differences clearly reflect the influence of cultural, economic, and geographical variables on consumption levels. Access to marine resources, income levels, and infrastructure are the primary drivers of these inequalities. Seafood market development is influenced by a variety of factors, including consumer preferences, sustainability concerns, and market integration. Developed countries frequently import high-quality seafood from developing countries due to market integration and efficiency (Asche et al., 2015). Consumer preferences for eco-labeled seafood can influence market dynamics; studies show that willingness to pay is a key factor in consumer preferences (Roheim et al., 2011). Understanding consumer segments and perceptions can help marketers develop effective strategies for specific markets. Studies focusing on luxury seafood in China (Wang and Somogyi, 2018) and research on preferences for eco-labeled seafood in Korea (Kim and Lee, 2018) highlight the importance of analyzing consumer behavior to tailor products to market demands. Studies on barriers to purchasing seafood in Australia highlight the importance of familiarity and preferences for specific seafood types among consumers (Birch et al., 2012). The Sustainable Seafood Movement is playing a significant role in reforming sustainability practices in seafood supply chains, influencing governance and market dynamics in the US and UK (Gutierrez and Morgan, 2015). Furthermore, developing local seafood markets can provide ecological benefits by reducing pressure on depleted stocks and minimizing bycatch waste (O'Hara and McClenachan, 2018). Understanding the interactions between small-scale fisheries and global aquaculture trade is critical for sustainable aquaculture practices and market development (Crona et al., 2016). The increasing demand for aquaculture products due to their health benefits, the recognition of aquaculture's nutritional value, and the market's growth have led to rapid trade development (Miao et al., 2021). Studies on the characteristics of freshwater fish markets in the world's largest aquaculture markets, such as China, have shed light on previously underexplored areas (Fang and Fabinyi, 2021). The aquaculture market has also been influenced by changing consumption patterns; an increasing portion of Chinese consumption comes from imported sources, underscoring the market's global importance (Fabinyi et al., 2016). The sustainability of aquaculture trade, as seen in the growth dynamics and sustainable development of exports between China and Vietnam, has been a focus for policymakers seeking to promote bilateral trade and formulate related policies (Wang et al., 2023). Consequently, the evolution of the seafood market is intricately intertwined with consumer preferences, sustainability initiatives, market integration, and governance practices. By considering these factors and leveraging insights from research on consumer behavior, sustainability movements, and market dynamics, stakeholders can contribute to the continued development of the seafood market while ensuring long-term sustainability.

5. Consumption Habits and Regional Differences in Turkey

Despite being surrounded by seas on three sides and possessing rich aquaculture potential, per capita aquaculture consumption remains below the global average. In 2019, per capita fishery consumption in Turkey was approximately 6.3 kg, while the global average was 8.2 kg (Sagun and Saygı, 2021; Can et al., 2015). This suggests that despite the country's geographical advantages, domestic consumption remains low. This discrepancy highlights the potential for increased aquaculture consumption in Turkey, particularly in coastal provinces where fishery products are more important to local diets. Seafood consumption habits in Turkey are influenced by various factors, including socioeconomic status, cultural preferences, and the availability of local seafood. Significant differences in consumption habits are observed between coastal regions and inland regions. In coastal regions such as the Black Sea, Marmara, Aegean, and Mediterranean, fish and seafood are consumed more frequently due to easy access to seafood and the influence of traditional culinary cultures. The Black Sea Region, in particular, contributes to high seafood consumption levels due to its robust fishing industry and cultural practices favoring fish dishes. Conversely, inland regions far from the coast, such as Central Anatolia, consumption levels are lower due to limited access to fresh seafood and differing dietary habits (Sagun and Saygı, 2021). Preferences among demographic groups also complicate the consumption landscape. For example, millennials in Turkey have significantly favored processed seafood over traditional wild fish, which can influence overall consumption trends (Güney and Sangün, 2017). This shift can be interpreted as a reflection of the pursuit of convenience and the shift toward processed foods brought about by modern lifestyles (Duyar and Güneri, 2024).

5.1. Factors Affecting Consumption and Health Awareness

Aquatic product consumption habits in Turkey are multifaceted and are influenced by health awareness, parental dietary habits, regional availability, childhood experiences, and national nutritional guidelines. Research has shown that increased health awareness, a heightened perception of the importance of nutrition, and healthy eating habits are positively correlated with seafood consumption (Birch et al., 2012). This reflects consumers' tendency to gravitate towards foods with high nutritional value. Parents' eating habits play a significant role in influencing children's seafood consumption (Govzman et al., 2020). Habits acquired during childhood largely shape individuals' dietary preferences in adulthood. Therefore, encouraging seafood consumption from an early age is critical for fostering healthy eating habits. Including fish and seafood consumption recommendations in national dietary guidelines is an important step toward raising this awareness (Sioen et al., 2007). However, despite Turkey's abundant fishing resources, the sector's largely export-oriented focus and limited awareness of seafood in the domestic market lead to low domestic consumption (Sagun and Saygı, 2021). This situation reveals that marketing and awareness strategies need to be developed to ensure that aquatic products find a wider place in the domestic market and reach consumers.

6. Market Dynamics and Development Potential

The Turkish aquaculture market has undergone significant transformations over the years, influenced by various factors such as the expansion of aquaculture, shifts in consumer preferences, and international trade dynamics. The transition from traditional capture fisheries to a more diversified aquaculture-based system has not only increased production but also necessitated adjustments to marketing and trade practices to meet both domestic and international demands. Consumer preferences have also evolved, leading to a significant increase in the consumption of processed aquaculture products. Research indicates that processed aquaculture products are particularly popular in Turkey's coastal regions, reflecting a broader trend seen in other countries, facilitating consumption patterns and increasing accessibility (Sagun and Saygı, 2021). The marketing structure of the aquaculture sector has adapted to these changes, with a dominant channel involving fishermen, brokers, retailers, and consumers (Kaygısız and Evren, 2019). This marketing structure has faced challenges, including issues related to traceability and consumer trust, which are increasingly important in the context of food safety and quality assurance (Tolon, 2017). The growth of Turkey's seafood exports demonstrates the country's positioning as a major player in the global market. Turkey exports a variety of seafood products, including fresh, frozen, and processed products, primarily to Europe and Asia (Mol et al., 2014). This demonstrates the international competitiveness and potential of Turkey's seafood sector. However, increasing domestic consumption and adopting sustainable production models are critical for the sector's more balanced and sustainable growth.

Consequently, seafood consumption in Turkey varies significantly by province, influenced by geographic, economic, and cultural factors. Coastal provinces exhibit higher consumption levels due to better access and cultural integration of seafood into local diets, while inland areas lag behind. Efforts to promote seafood consumption could further enhance these trends and align Turkey's consumption levels more closely with global averages.

7. Nutritional Benefits of Seafood

Seafood is a valuable food group, containing many nutrients essential for human health. Their high-quality protein content, in particular, is a fundamental building block for muscle growth and repair. Fish and seafood contain all the essential amino acids that the body cannot produce on its own and must be supplied externally (FAO, 2022). This makes seafood an important source of protein, especially for those seeking alternatives to vegetarian or vegan diets. One of the most well-known benefits of seafood is its richness in omega-3 fatty acids (EPA and DHA). These fatty acids play a critical role in maintaining cardiovascular health, improving brain function, and reducing inflammation (Kris-Etherton et al., 2009). Regular omega-3 intake may reduce the risk of heart attack and stroke, lower blood pressure, and balance triglyceride levels (Mozaffarian and Wu, 2011). It also contributes to brain and eye development in children, may support cognitive functions in adults, and may reduce the risk of depression (Swanson et al., 2012). Seafood is also rich in vitamins. Vitamin D, in particular, is vital for bone health, immune system function, and overall metabolism. In regions where sunlight exposure is limited or during the winter months, fish consumption can be an important source of vitamin D (Holick, 2007). Vitamin B12 is essential for nervous system health and red blood cell production; seafood is one of the most natural and bioavailable sources of this vitamin (O’Leary and Samman, 2010). In terms of minerals, seafood contains important elements such as iodine, selenium, zinc, and phosphorus. Iodine is essential for the production of thyroid hormones and has a direct effect on metabolism and growth (Zimmermann, 2009). Selenium, a powerful antioxidant, protects cells from damage and supports the immune system (Rayman, 2012). Zinc is important for immune function, wound healing, and DNA synthesis (Prasad, 2008). Phosphorus plays a role in bone and dental health, as well as energy metabolism (Calvo and Tucker, 2013).

7.1. Risks and Safety Precautions of Seafood Consumption

While the health benefits of seafood are undeniable, there are also some potential risks. Chief among these risks are contaminants such as methylmercury, which can accumulate, especially in large, long-lived fish (Fujimura and Yoshinaga, 2023). Methylmercury can have toxic effects on the nervous system and pose a particular risk to pregnant women, nursing mothers, and young children (Duyar et al., 2023). Therefore, national and international health organizations issue recommendations regarding the consumption of certain fish species. For example, consumption of species such as swordfish, shark, king mackerel, and tilefish should be limited (FDA, 2019). Other food contaminants, such as heavy metals (cadmium, lead), pesticides, and phytotoxins, can also enter the human body through seafood (Yap and Al-Mutairi, 2022; Duyar et al., 2023; Daş et al., 2009). The accumulation of these contaminants can lead to various health problems in the long term. To minimize these risks, it is crucial to procure seafood from reliable sources, effectively implement traceability systems, and conduct regular inspections (Tolon, 2017). Another risk associated with seafood consumption is foodborne infections. Raw or undercooked seafood can harbor pathogens such as bacteria (Salmonella, Vibrio), viruses (Norovirus), and parasites (Anisakis) (Choudhury et al., 2022). These pathogens can cause serious illness, especially in individuals with weakened immune systems. Therefore, proper cooking of seafood, preventing cross-contamination, and adhering to hygiene rules are vital. When consuming products containing raw fish, such as sushi and sashimi, utmost care should be taken to ensure the freshness and safety of the product (Centers for Disease Control and Prevention (CDC), 2022). Seafood allergies are also a significant health problem. Fish and shellfish are potent allergens that can cause severe allergic reactions (anaphylaxis) in some individuals (Rama and Silva, 2022). These allergies typically appear in childhood and can persist throughout life. Individuals with allergies should strictly avoid products containing allergens and carefully read label information (Khora, 2020).

7.2. Recommendations for Safety and Sustainability

Several precautions should be taken to maximize the health benefits of seafood consumption while minimizing risks:

Variety: Consuming a variety of fish types and sizes, rather than relying on a single species, can help reduce potential contaminant accumulation. Smaller, shorter-lived fish generally contain less mercury (FDA, 2019).

Source Information: Consumers should learn about the source, catch, or farming methods of the seafood they purchase. Products with sustainable fishing certifications (such as MSC) should be preferred (Carlucci et al., 2015).

Cooking Methods: Seafood should be thoroughly cooked until internal temperatures reach safe levels. Raw or undercooked consumption should be avoided (Burns et al., 2023).

Storage and Hygiene: Seafood should be quickly cooled after purchase and stored under appropriate conditions. To prevent cross-contamination, raw seafood should be kept separate from other foods (U.S. Food and Drug Administration (FDA), 2020). Information and Education: It is important to inform consumers about the nutritional benefits, potential risks, and safe consumption methods of seafood. Public service announcements, educational campaigns, and nutritional counseling can be effective in this regard (Partelow et al., 2023).

Global events such as the COVID-19 pandemic have caused disruptions in seafood supply chains and changes in consumption patterns (Villasante et al., 2021). Such crises have once again highlighted the importance of food safety and sustainability. International cooperation and flexible supply chain management strategies should be developed to create an aquaculture system more resilient to future crises (Bonanomi, et al., 2017). Seafood consumption plays a critical role in individual health and global food security. To maximize benefits while minimizing risks, making informed choices, choosing reliable sources, and using appropriate preparation methods are essential. This approach will both protect the health of consumers and support the sustainability of marine ecosystems.

8. Factors Influencing Consumer Behavior

Seafood consumption trends vary according to various factors, including demographics, income level, education, and cultural preferences. Research in the United States has revealed that age, income, and education level significantly influence seafood consumption (Jahns et al., 2014). Rising income and purchasing power, in particular, are driving global demand for seafood, particularly in developing economies (Guillen et al., 2019). The US seafood consumption pattern is shifting toward a concentration on a few main species, with a potential increase in imported products if the decline in certain species, such as canned tuna, continues (Shamshak et al., 2019). Furthermore, seafood consumption is not limited to home-cooked meals, as fast food chains and restaurants play a significant role in the American seafood diet (Love et al., 2020). In the US, a significant portion of seafood consumption by weight and expenditure occurs outside the home, particularly in restaurants (Love et al., 2021). This highlights the importance of considering both in-home and out-of-home consumption patterns when analyzing seafood consumption trends. Sustainability concerns are increasingly shaping seafood consumption habits, especially among younger generations. Research suggests that Generation Z consumers are more likely to consider the environmental impact of their seafood choices, which may influence their purchasing decisions (Gibson et al., 2023). This increased awareness of sustainability issues is critical as it aligns with global efforts to promote responsible seafood consumption and address overfishing and environmental degradation (Cisneros-Montemayor et al., 2016).

8.1. Efforts to Promote Sustainable Consumption

Efforts are underway to promote sustainable seafood consumption by focusing on factors such as conservation, responsible production, and healthy eating (Farmery et al., 2021). Sustainable seafood industries are being promoted through recommendations to increase domestic consumption of US-produced seafood to support sustainability (Gephart et al., 2019). Furthermore, interventions aimed at improving seafood consumption habits, such as promoting aquatic lifestyles and appropriate product varieties, can be effective in increasing seafood consumption (Birch et al., 2018). The safety and health impacts of seafood consumption are crucial. Given the risks associated with contaminants such as methylmercury, awareness of seafood safety is crucial (Fujimura and Yoshinaga, 2023; Duyar et al., 2023). Furthermore, the nutritional benefits of seafood, such as its high omega-3 content, are well recognized, but the quality and source of seafood consumed can influence health outcomes, as seen in the association between seafood rich in n-3 PUFAs and reduced fatty acids (Zeng et al., 2023). Determinants of seafood consumption are influenced by various factors, including socio-demographic characteristics, health awareness, and cultural preferences (Choudhury et al., 2022; Govzman et al., 2020; Pupavac et al., 2022). Studies suggest that understanding the sustainability and environmental impacts of seafood may influence consumer preferences for eco-labeled seafood, reflecting increased awareness of the negative impacts of traditional seafood production methods (Winson et al., 2021; Kim and Lee, 2018). Additionally, concerns about seafood safety and willingness to pay for seafood safety assurances reflect a shift toward more sustainable consumption practices (Wessells and Anderson, 1995). The COVID-19 pandemic has also impacted seafood consumption patterns, with disruptions in supply chains and shifts in demand impacting the seafood sector globally (Villasante et al., 2021). However, efforts to raise awareness of the benefits of seafood consumption, such as its role in nutrition and health, are ongoing (Partelow et al., 2023). Seafood consumption in general is a complex behavior influenced by a combination of cultural, social, economic, and health factors. Understanding these influences is crucial to promoting sustainable and healthy seafood consumption practices worldwide.

8.2. Global Consumption Habits and Sustainability

Aquatic product consumption habits worldwide are influenced by a variety of factors, including cultural traditions, health perceptions, economic conditions, and environmental sustainability concerns. Global average seafood consumption has reached approximately 20 kg per person per year, with significant variation across regions and demographics (Birch et al., 2018). For example, countries such as Portugal and Japan exhibit significantly higher seafood consumption, reflecting deep-rooted cultural practices and dietary preferences, with individuals consuming seafood four to five times per week (Almeida et al., 2015). It is important to develop effective strategies to promote healthier and more sustainable seafood consumption practices globally.

Aquatic product consumption habits in Turkey are influenced by a variety of factors, as evidenced by research. Research has shown that health awareness, perceptions of the importance of nutrition for health, and healthy eating habits are positively associated with seafood consumption (Birch et al., 2012). Additionally, parental dietary habits play a significant role in influencing seafood consumption in children (Govzman et al., 2020). Turkey, home to significant aquatic resources in the Black Sea, Marmara, Aegean, and Mediterranean regions, is encouraged to increase its consumption (Sagun and Saygı, 2021). However, despite Turkey's abundant fisheries, domestic consumption remains low due to the sector's focus on exports (Sagun and Saygı, 2021). National dietary guidelines include recommendations for consuming fish and seafood due to the positive health effects of seafood consumption (Sioen et al., 2007). However, awareness of seafood safety issues is important, as seafood consumption has been linked to foodborne outbreaks in various regions (Choudhury et al., 2022).

9. Market Structure and Development

The seafood market is a key component of the global food system and has evolved over time. Initially, it was heavily dependent on capture fisheries, which provided a significant portion of the global aquaculture supply. However, the rapid growth of aquaculture has fundamentally changed the market landscape. Aquaculture is now considered one of the fastest-growing sectors in food production and contributes significantly to the global aquaculture supply (FAO, 2022). This transition has not only increased the volume of aquaculture produced but also transformed production practices, making it a critical component of the aquaculture economy of many countries. For example, the expansion of intensive aquaculture in countries like Turkey has enabled the diversification of aquaculture products available in both domestic and export markets. Turkey exports a variety of seafood products, including fresh, frozen, and processed products, primarily to European and Asian markets (Mol et al., 2014). This clearly demonstrates the impact of aquaculture on global trade dynamics. Seafood market development is influenced by various factors, including consumer preferences, sustainability concerns, and market integration. Developed countries frequently import high-quality seafood from developing countries for reasons of market integration and efficiency (Asche et al., 2015). This highlights the complexity of global supply chains and the importance of international trade.

9.1. Trade Dynamics and Global Integration

International trade in aquaculture products is crucial for global food security and economic development. Global aquaculture trade has reached a volume of billions of dollars and contributes significantly to the economies of many countries. This trade dynamics are influenced by factors such as the demand for sustainable aquaculture products and the implementation of traceability systems aimed at increasing consumer confidence in aquaculture products (Tolon, 2017; Lu et al., 2022). The rise of eco-labeling and sustainability movements has also influenced consumer preferences, with a growing segment of the market willing to pay a premium for sustainably sourced aquaculture products (Roheim, et al., 2011; Kaba and Duyar, 2008).

This suggests that the market is shifting toward more sustainable products, driven by increasing consumer awareness of environmental and social responsibility. Encouraging trade measures that distinguish between sustainable and unsustainable fish products can encourage sustainable fish production and consumption (Baumgartner and Bonanomi, 2021). By providing preferential commercial treatment for sustainably sourced fish, the market can accelerate the transition to more sustainable fishing practices. The development of the aquaculture market has been influenced by a variety of factors over the years. Both domestic and international consumer demand has been a significant driver for aquaculture businesses to innovate technologically and improve production methods, increasing the competitiveness of aquaculture products in the global market (Huang et al., 2023). Concerns about food safety and quality have also played a significant role in shaping the industry, influencing consumer perceptions, and influencing the development of aquaculture markets (Cui et al., 2022).

9.2. Market Challenges and Opportunities

The aquaculture market, despite its dynamic nature, also faces various challenges. These include the impacts of climate change on fish stocks, illegal, unreported, and unregulated (IUU) fishing, supply chain disruptions, and food fraud. These challenges threaten the stability and sustainability of the market. For example, food fraud not only erodes consumer trust but also creates unfair competition for businesses operating legally and ethically (Fox et al., 2018). Despite these challenges, significant opportunities exist in the aquaculture market. Increasing global demand offers potential for further development of aquaculture. Innovative production technologies are enabling the development of more efficient and environmentally friendly aquaculture systems. Furthermore, developments such as digitalization and blockchain technologies can improve food safety and transparency by increasing traceability throughout the supply chain (Kruk et al., 2023). Efforts to improve food safety systems, both through government oversight and private sector incentives, have been emphasized as essential to ensure market access and increase the effectiveness of food safety measures in international trade of seafood (Liu et al., 2012). Furthermore, innovations in preservation techniques, such as the development of natural preservatives, have addressed concerns about spoilage and food safety by extending the shelf life of seafood and preserving their quality (Feng et al., 2023; Duyar et al., 2020; Lu et al., 2022).

Consequently, the seafood market has evolved significantly over the years, driven by factors such as consumer demand, food safety concerns, market characteristics, and sustainability considerations. Understanding these dynamics is crucial for stakeholders in the sector to overcome challenges and capitalize on further growth and development opportunities. Future growth must be supported by the adoption of sustainable practices, technological innovations, and global collaboration.

10. Cultural and Social Influences

Seafood consumption habits are shaped by profound cultural and social factors worldwide. Traditional practices and cultural heritage play a significant role in determining individuals' preferences for seafood (Govzman et al., 2020). Religious and cultural traditions can positively influence seafood consumption by encouraging certain dietary habits. For example, halal dietary laws in Muslim countries directly influence seafood consumption patterns (Wilson and Liu, 2010). Understanding the role of halal certification is critical to understanding consumption habits in these communities. In countries with high fish consumption, such as the Pacific Islands, seafood allergies are common due to cultural practices of consuming raw or undercooked seafood (Rama and Silva, 2022). This demonstrates the potential impact of cultural habits on health. In developed countries such as Australia and the United States, seafood consumption is often shaped by habitual behaviors formed during childhood. Research suggests that individuals who regularly consume seafood as children are more likely to continue these habits into adulthood (Christenson et al., 2017; Neale et al., 2012). However, barriers such as lack of familiarity, cooking confidence, and perceived difficulty of preparing seafood can inhibit consumption. For example, in Australia, a significant portion of the population does not actively remember to include seafood in their diets, suggesting they rely on habitual food preferences (Birch et al., 2012; Birch and Lawley, 2013). This habitual nature of seafood consumption suggests that interventions aimed at increasing familiarity and cooking skills can effectively increase intake levels (Burns et al., 2023).

11. Economic Factors and Accessibility

Economic factors also play a significant role in seafood consumption patterns. In the United States, seafood purchasing behavior varies by income level, with higher-income individuals tending to consume more seafood (Jahns et al., 2014; Hoffman et al., 2021). This is due to the fact that seafood is generally more expensive than other protein sources. Increased income levels facilitate consumers' access to a wider variety of high-quality seafood (Guillen et al., 2019). Global events such as the COVID-19 pandemic have significantly impacted consumption patterns. Many consumers have chosen to prepare seafood at home instead of eating out, leading to an increase in the consumption of more accessible seafood options (White et al., 2020; Engle and Nyre. (2023). This shift demonstrates how quickly consumer behavior can adapt to external conditions. Furthermore, the price and availability of seafood have a direct impact on consumption, especially in rural and inland areas. Easier access to fresh seafood in coastal areas is one of the main reasons for higher consumption rates (Sagun and Saygı, 2021).

12. Health Awareness and Environmental Concerns

Health awareness is a significant factor influencing seafood consumption. As consumers learn that seafood is rich in nutrients such as omega-3 fatty acids, protein, and vitamins, their demand for these products increases (Choudhury et al., 2022). National dietary guidelines also support this awareness by encouraging the consumption of fish and seafood (Sioen et al., 2007). However, concerns about contaminants such as methylmercury may cause some consumers to limit their seafood consumption (Fujimura and Yoshinaga, 2023). Therefore, accurate information about safe consumption amounts and types is crucial. Environmental concerns are increasingly shaping seafood consumption habits, especially among younger generations. Research shows that Generation Z consumers are more likely to consider the environmental impact of their seafood choices, which can influence their purchasing decisions (Gibson et al., 2023). This growing awareness of sustainability issues is critical as it aligns with global efforts to promote responsible seafood consumption and address overfishing and environmental degradation (Cisneros-Montemayor et al., 2016). Eco-labeling and certification programs help consumers choose products from sustainable sources (Carlucci et al., 2015; Kaba and Duyar, 2008).

Consequently, seafood consumption habits are multifaceted and influenced by cultural, economic, health, and safety factors. Understanding these dynamics is essential to promoting sustainable and healthy seafood consumption practices globally. Consumer education, increased accessibility, and the dissemination of sustainable production methods are key to progress in this area.

13. Assessment of Global and Local Consumption Dynamics

Seafood consumption is a multidimensional phenomenon resulting from the complex interaction of cultural, economic, environmental, and health-related factors worldwide. Its health benefits, richness in omega-3 fatty acids, and low fat content make it a significant part of individual dietary habits. However, consumption rates vary significantly across countries, regions, age groups, and income levels. While Asian countries lead in per capita consumption due to their traditional culinary cultures and high production capacities, some African and Middle Eastern countries lag behind due to low income levels and access restrictions. In Turkey, it is striking that per capita seafood consumption remains below the global average despite being surrounded by sea on three sides. The higher consumption rates in coastal regions compared to inland regions clearly demonstrate the influence of ease of access to seafood and cultural habits. However, the sector's predominant focus on exports and limited awareness of seafood in the domestic market are key factors limiting domestic consumption. This situation reveals that Turkey cannot fully utilize its aquaculture potential and that the domestic market needs to be developed.

14. Sustainability and Consumer Responsibility

Sustainability has become a global imperative. The growing population and demand for seafood are placing significant pressure on natural fish stocks and threatening the health of marine ecosystems. In this context, sustainable fishing and aquaculture practices are evolving as a paradigm encompassing not only environmental but also economic and social dimensions. Sustainable fishing aims to protect the renewability of fish stocks and the health of marine ecosystems, while sustainable aquaculture aims to ensure efficient production while reducing the environmental footprint. Consumer responsibility plays a key role in achieving sustainability goals. Consumers' preference for eco-labeled products, their willingness to pay a premium for products from sustainable sources, and their knowledge of seafood encourage sustainable consumption practices (Carlucci, et al., 2015; Winson, et al., 2021).

To ensure sustainability in fish consumption, several key factors must be considered. Consumer perceptions and beliefs play a crucial role in promoting sustainable fish consumption (López-Mas et al., 2023). Understanding how consumers view sustainability can help develop effective strategies to promote the consumption of sustainably sourced fish. Additionally, increasing consumer familiarity with eco-labels has been identified as a way to promote sustainable fish consumption (Santos and Pereira, 2021). The sustainability of fish consumption is closely linked to aquaculture practices. Aquaculture's dependence on fish caught for fishmeal and fish oil production highlights the need for aquaculture to mitigate this dependence to ensure sustainability (Guillen et al., 2019). Optimizing fish utilization for human consumption is another important aspect of sustainability (Bogard et al., 2019). Increasing fish consumption for animal feed can lead to greater consumption of nutritious and sustainable aquaculture products without the need to increase catches (Farmery et al., 2021). Furthermore, the role of trade measures in distinguishing between sustainable and unsustainable fish products can help increase sustainable fish production and consumption (Baumgartner and Bonanomi, 2021). Drawing clear lines between sustainable and unsustainable fish products is important to support sustainable development. Furthermore, addressing factors such as supply adequacy, safety, social and economic sustainability, and system resilience are crucial to ensuring the sustainability of fish consumption (Jennings et al., 2016).

15. Recommendations

The following recommendations are offered to maximize both the health benefits of seafood consumption and ensure environmental sustainability:

15.a. Education and Awareness Campaigns: National-wide education and awareness campaigns should be organized to inform consumers about the nutritional benefits of seafood, safe consumption methods, and sustainable sources. These campaigns should contribute to the development of healthy seafood consumption habits, especially starting in childhood.

15.b. Supporting Eco-labeling and Certification Systems: Eco-labeling and certification systems (e.g., MSC) should be expanded and supported to enable consumers to easily identify seafood from sustainable sources. These labels will help consumers make informed choices and encourage sustainable fishing practices.

15.c. Developing Policies for the Domestic Market: In export-oriented countries like Turkey, strategies should be developed to increase domestic consumption. This could include policies such as tax reductions, subsidies, or marketing supports to encourage local producers to focus more on the domestic market. Additionally, it is important to make seafood more accessible and affordable at retail outlets.

15.d. Increasing Food Safety and Traceability Measures: To increase consumer confidence in seafood, food safety standards should be raised, and traceability systems should be strengthened throughout the supply chain. This should apply to both local and imported products and minimize potential contamination or fraud risks.

15.e. Promoting Sustainable Production and Aquaculture: Sustainable fishing quotas and methods should be implemented to prevent overfishing and protect natural fish stocks. Integrated and innovative production techniques (e.g., IMTA) that reduce the environmental footprint should be promoted in the aquaculture sector. This will both ensure the sustainability of production and protect the health of marine ecosystems.

15.f. Research and Development: Continuous research and development activities should be supported on seafood consumption habits, market dynamics, and sustainability. This research will help identify new consumption trends, understand market needs, and develop more effective sustainability strategies. Implementing these recommendations will strengthen the role of aquaculture in global food security and ensure the long-term health of marine ecosystems. With informed consumers, responsible producers, and supportive policies, the aquaculture sector can move toward a more sustainable and prosperous future.

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CHAPTER 6

EARLY STAGE STOMACH pH MEASUREMENT IN ALTRICIAL FISH LARVAE; FLUORESCENT POWDER FEED PROBES

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1. INTRODUCTION

The highest losses in fish production occur in the larval stages. This situation is directly related to the physical-chemical quality of the water as well as the larval feeding being done in accordance with the physiological changes in the early stage. From this point of view, it is very important to monitor the developmental stages of fish larvae well; to reduce early larval losses and to determine the development of the digestive system up to the post-larval stage and to make effective feeding practices. Insufficient stomach acid grade, low enzyme level and not fully developed digestive system necessitate absorption of ingested nutrients mostly through mechanical digestion in the early life stage of fish. This prevents digestion of artificial powder baits. Functional stomach is formed with the acid level in stomach becoming suitable for digestion, enzyme activity increasing and differentiation of digestive tract. Up to this stage, various zooplankton (Artemia sp., *Brachionus plicatilis* and infusoria etc.) in sizes suitable for the size of the mouth gap of larvae are used.

Especially in intensive commercial production, the highest cost in hatcheries is incurred during the use of live feeds in larval feeding applications. The sooner larvae are transitioned to granulated feeds, the lower the cost burden. This is related to determining the earliest day the larvae develop a functional stomach and discontinuing zooplankton use at the most appropriate time. Determining the appropriate levels of stomach acid required to digest granulated feeds is crucial in this regard.

Larval ontogeny and functional stomach formation

Studying tissue and organ formation during fish larval development generally requires the use of microscopic and histological methods. These methods are essential tools for determining early larval feeding strategies, monitoring metamorphosis, and identifying key developmental stages such as mouth opening, swim bladder development, morphological differentiation of the digestive system, and the formation of a functional stomach. In this context, histological analyses play a critical role in understanding the developmental biology of larvae (Sepil et al., 2025). Monitoring the development of the stomach, digestive tract and digestive enzyme levels such as amylase, pepsin, and trypsinogen is important both for determining the early stage feeding protocol and for monitoring functional stomach formation.

In teleost fish, the most important indicator that marks the completion of the transition from larval to juvenile stages is the development of a fully functional adult digestive system. Although the digestive system is generally immature during the first feeding period, it is still rudimentary in altricial marine fish larvae (Falk-Petersen, 2005; Yúfera and Darias, 2007). In the early stage, digestion occurs primarily in the intestine under alkaline-neutral pH conditions, with the contribution of cytosolic enzymes derived from the pancreas and present in enterocytes. Protein digestion is primarily driven by the action of the alkaline proteases trypsin and chymotrypsin, as well as cytosolic peptidases (Zambonino-Infante and Cahu, 2001). Digestive function is one of the key factors in the early-stage survival strategy of larval teleosts. Pepsin is believed to be responsible for the digestion and partial hydrolysis of protein in the stomach, which is completed in the intestine by the combined action of trypsin and chymotrypsin. Pepsins are present in the stomach of many teleosts, and pepsinogen-secreting glands are present in the esophagus of some fish. Early-stage larvae are unable to digest dry pellets because these enzymes have not reached a sufficient level of activation (Cahu and Zambonino-Infante, 1994; Lo and Weng, 2006).

The formation and functioning of gastric glands in larvae vary depending on the fish species, and they secrete pepsinogen and hydrochloric acid, which lower the pH in the stomach and initiate the conversion of pepsinogen to pepsin. The presence of gastric glands in larvae does not indicate the organ's full functionality. An increase in enzyme activity alone is not sufficient for the formation of a functional stomach. This is because acidity is directly related to the activation of most enzymes. Pepsin activity, in particular, and the acidification necessary for its activity are also essential (Yúfera et al., 2004).

Considering all these interconnected changes, determining the actual time of stomach formation in larvae, which varies by species, is related to enzyme activity, histological differentiation in the stomach, foregut, midgut and hindgut, and monitoring changes in acidity. Acidity is the most important parameter affecting all important developmental processes, such as digestive system metamorphosis, the functionalization of relevant glands, and increased enzyme activity.

Use of Anthocyanin and Other pH Indicator Solutions

Anthocyanins constitute the most important group of flavonoid pigments. Although the term "anthocyanin" was first used in 1835, the pH indicator properties of these compounds have been known since 1664 (Harborne and Williams, 2001; Keleş, 2015). The first structurally identified anthocyanin was isolated from the blue-flowered plant *Centaurea cyanus* in 1913. To date, the number of anthocyanin compounds with identified structures has exceeded 600. Flavonoids, including anthocyanins, have diverse functions in plants. These functions include signaling to microorganisms, defense against pathogens, increasing tolerance to biotic and abiotic stress conditions, regulating auxin transport, promoting plant productivity, and making flowers more visible to insects and other animal pollinators. Furthermore, anthocyanins play an important role in the diversity of flower colors in angiosperms (Schwinn and Davies, 2004; Keleş, 2015).

Anthocyanins are pigments in the flavonoid group, belonging to the class of phenolic compounds, which are one of the secondary plant metabolites (Barros et al., 2016; Onan and Çölgeçen, 2023). Widely found in plants and considered one of the most important pigment groups, anthocyanins are water-soluble compounds that impart pink, orange, red, purple, and blue colors to various plant parts such as roots, stems, leaves, flowers, fruits, and seeds (Kong et al., 2003; Onan and Çölgeçen, 2023).

Chemically, anthocyanins are glycosides of polyhydroxy and polymethoxy derivatives of 2-phenylbenzopyrylium salts, and due to their structural properties, the color of anthocyanins changes reversibly with pH. The red flavylium cation predominates in the pH range of 1–3, the colorless carbinol pseudobase and chalcone forms in the pH range of 4–5, and the blue quinoidal anhydrobase form above pH 5 (Koh et al., 2020; Onan and Çölgeçen, 2023). Naturally occurring anthocyanins in plants are generally in glycosylated form. These glycoside structures contain sugar units such as glucose, galactose, arabinose, rhamnose, or xylose attached to the anthocyanidin skeleton (Huang and Zhou, 2019; Onan and Çölgeçen, 2023).

Anthocyanins are also widely used in the food industry. Recent studies have explored the composition, intended uses, and food industry applications of indicator smart films prepared using anthocyanins. Smart films containing limonene, developed by Liu et al. (2017), showed a color change from pink to red in pasteurized milk after 48 hours. This color change provides a visual indicator of the product's spoilage process. Similarly, anthocyanin-containing colorimetric films developed by Zhai and colleagues (2017) were used to detect the accumulation of amines released as a result of spoilage in seafood. These films provide visual information about product freshness by transforming anthocyanins from pink-purple to blue (Takma and Nadeem, 2019).

Anthocyanins are non-toxic compounds found naturally in many fruits and are suitable for use in studies on fish larvae (Figure 1). Additionally, various indicators such as bromphenol blue, thymol, and bromcresol green, which change color depending on pH, can also be used. Of these, bromphenol blue, like anthocyanins, produces a very wide color spectrum depending on acidity (Figure 2).

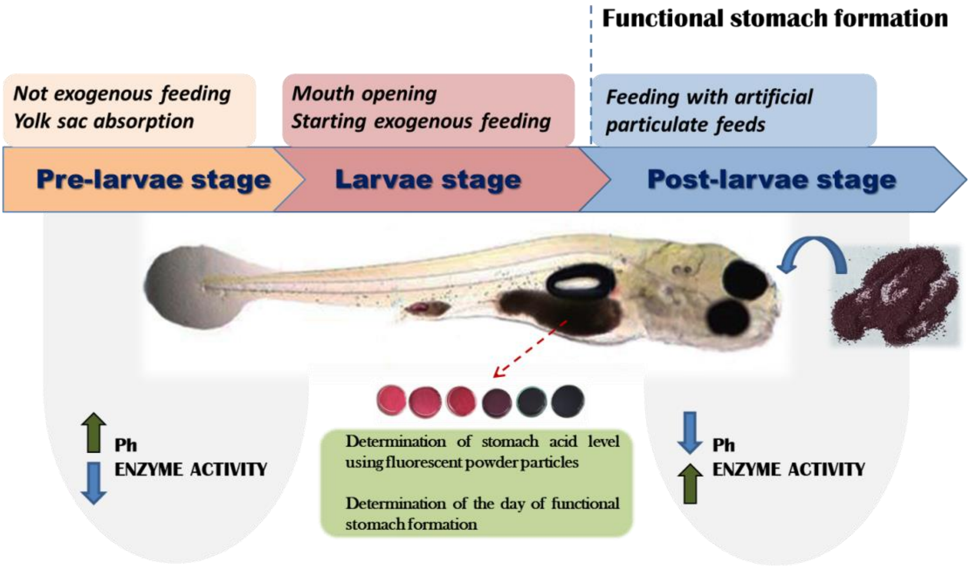

Figure 1. Use of pH indicators in early larval stages.

Anthocyanins and other pH-indicating compounds can be used in artificial fish larval diets by imbibing them with commercially available powder particles prepared to fit the larvae's mouth opening (Figure 3). In this case, leaching of the imbibed anthocyanin into the water may occur during the time the larvae ingest the food. However, since the desired goal is to deliver even a small amount of fluorescent material to the larvae and cause a color change in the digestive tract, particularly the stomach, the amount of anthocyanin entering the larvae's digestive tract is not critical.

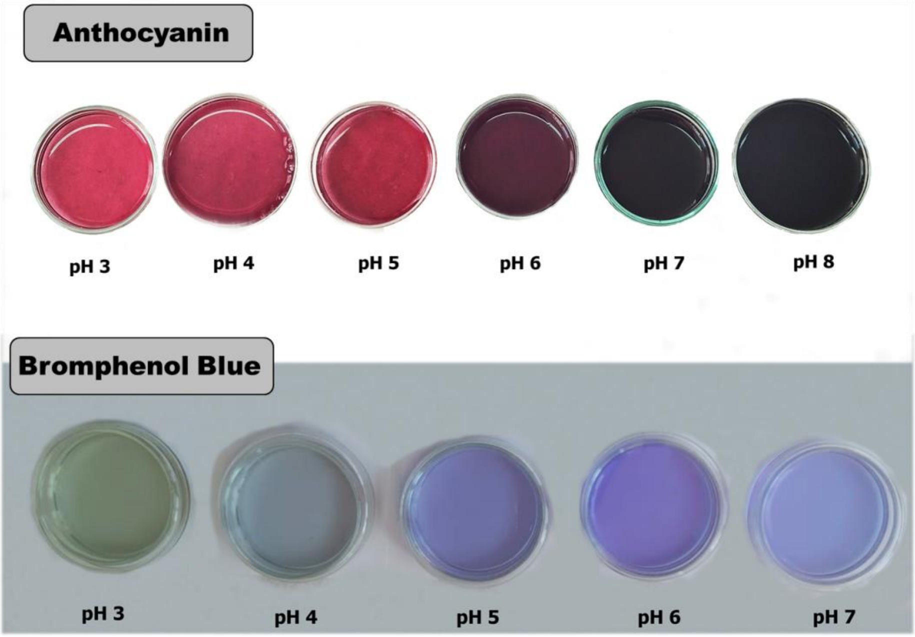

Figure 2. Color changes of anthocyanin and bromphenol blue in waters with different pH values.

If it is intended to prevent the leakage of anthocyanin or other indicators until they are ingested by the larvae, the baits can be encapsulated using substances that the larvae can digest or coated with gelatin-alginate at this stage.

In this study, feed particles were prepared by pulverizing commercial feeds, with an average size of 14.42±3.21 µm (n: 60), suitable for the first mouth opening of altricial fish larvae. The particulate feeds were placed in glass petri dishes and covered with a solution obtained by dissolving anthocyanin oxalates in distilled water. The baits were then incubated at 30°C for 24 hours to allow the feeds to absorb the fluorescent solution.

Figure 3. Artificial feed particles prepared by impregnating anthocyanin.

Analyzing color tones and determining pH levels

Samples of cultured altricial fish larvae should begin the day following mouth opening, i.e., exogenous feeding. At this stage, at least 10 larvae should be placed in 100-250 mL glass beakers at the same time each day. Using reverse osmosis water or distilled water at the same temperature as the larval stock tank in the beakers will reduce suspended particles. However, agitating the water in the beakers with an air stone strong enough to not hinder the free swimming movement of the larvae will ensure that the fluorescent baits added to the water are continuously distributed within the water column and that the larvae can reach the baits more easily.

After adding food particles to the larvae in beakers (3-5 larvae/beaker), it's advisable to wait a short time for all larvae to ingest the probe food before examining the samples under a microscope. This time should be no longer than 10-15 minutes, so the powder particles can be tracked throughout the digestive tract before they are fully absorbed by the larvae.

After the larvae ingest the prepared fluorescent feeds, larval samples that are still transparent and have not yet developed body pigmentation will show color changes depending on the acidity level in the stomach and digestive tract. The most important point at this stage is to photograph color reference samples from waters with different pH values within the same frame while capturing images of the digestive tract of the live larva under a microscope. In other words, the color reference waters prepared using the fluorescence in the larvae's stomachs must also be at the same magnification and light intensity. The RGB (red, green, blue) values of the obtained reference colors were determined using Image J software, and stomach pH levels were determined by comparing them with the RGB values of the color change observed in the stomach of the larvae in microscope images (Figure 4). At this stage, the similarity and statistical differences between the RGB values of the colors (reference and stomach) were evaluated using SPSS software.

RGB Value
Label,Area,Mean,Min,Max
1,Red,5788,121.484,119,124
2,Green,5788,132.332,130,134
3,Blue,5788,140.920,137,143
4,(R+G+B)/3,5788,131.703,129,133
5,0.299R+0.587G+0.114B,5788,129.997,128,131

Figure 4. Determining RGB values of color changes.

2. CONCLUSION

It is clear that a better understanding of larval feeding and digestive physiology will contribute to the optimization of diet preparation and feeding protocols and even improve early-stage survival rates in many common aquaculture species. Designing diets that meet the requirements of effective larval feeding, digestion, and absorption requires an integrated understanding of the various factors and events that interact during food acquisition and digestion. Crucially, better knowledge in this area will translate into reduced larval production costs (Rønnestad et al., 2013).

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CHAPTER 7

GLOBAL TRENDS IN CROP PROTECTION INDUSTRY

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1. Population Growth, Cereal Production and Consumption, and Climate Change

Projections from the Food and Agriculture Organization of the United Nations (FAO, 2024) indicate that the world's population will surpass 10 billion by 2050. One of the most pressing challenges associated with this demographic surge is ensuring sufficient food production to meet the growing population's needs. Although, in principle, global agriculture has the potential to provide sufficient food for all, the latest State of Food Security and Nutrition in the World (SOFI) report (FAO, 2023) reveals that around 733 million people still have been faced with hunger. The primary drivers of this situation include ongoing conflicts, abnormal climatic conditions, and economic shocks. The continuing global climate emergency has intensified challenges related to food security, serving as a major driver of hunger and widespread food-related crises. It is particularly noteworthy that agriculture is one of primary contributors to climate change. At the same time, this sector is acutely susceptible to the negative consequences brought about by shifting environmental conditions (FAO, 2024). Projections for the 2025/26 season indicate that global wheat production is set to reach to maximal production level. Despite this anticipated increase, the downward trend in global wheat stocks observed in recent years is expected to persist. Since the 2015/16 season, global wheat stocks have ranged between 250 and 280 million metric tons, although a noticeable decline has occurred since the 2021/22 season. By 2025/26, total stocks are expected to remain near 250 million metric tons (Sowell, 2025).

An analysis of the geographical distribution of these stocks reveals that China holds the largest share of global wheat reserves, followed by India. The principal exporting nations—Argentina, Australia, Canada, the European Union, Kazakhstan, Russia, Ukraine, and the United States—together hold only a modest share of the world's total grain reserves. Countries outside the primary exporters are collectively classified as the "rest of the world." This classification underscores the crucial roles of China and India in ensuring stability within the global wheat supply. In recent years, declining stock levels among major exporting nations have been a key factor driving greater fluctuations in both international wheat trade and pricing. Although production is expected to rise in the 2025/26 season, persistently low reserves suggest that fragility in the global wheat market may continue (Sowell, 2025).

Fluctuations in the global wheat market are not solely attributable to production and stock levels. Disruptions to the supply chain, which initially emerged as a result of the COVID-19 pandemic and were subsequently exacerbated by the effects of climate change, have become even more complex due to the conflict between Russia and Ukraine, as well as persistent political frictions involving India and China. Additionally, recent economic downturns have further contributed to the volatility observed in the wheat market. The steadily growing world population continues to drive up wheat demand, pushing the supply-demand balance to increasingly delicate levels.

The pandemic-induced surge in demand for staple foods has notably increased the consumption of durable wheat products such as pasta and bulgur, providing a significant advantage for wheat-exporting countries. Conversely, the Israel-Palestine conflict, subsequent Houthi attacks in Yemen, and disruptions to maritime trade in the Red Sea have introduced new obstacles to global wheat trade. The necessity for commercial vessels to take longer routes has led to higher costs and extended delivery times. According to a report by the World Trade Organization, the volume of wheat passing through the Suez Canal has declined, adversely affecting wheat trade from the EU, Russia, and Ukraine to East Africa and Asian countries (Keykubat & Kişi, 2024).

Ensuring global food security now have been depended more than ever upon the careful management of agricultural resources. The sector is under mounting pressure from climate change, which has introduced a host of environmental stressors. Higher average temperatures, extended periods of drought, flooding, soil salinity, and diminishing water supplies have all begun to threaten both the stability and quality of crop yields. Developing countries, in particular, face heightened vulnerability in this context.

Greenhouse gas emissions, largely the result of human activities, have intensified, causing global temperatures to rise and increasing the frequency of extreme weather events. This trend has gradually eroded the resilience of agricultural systems. Since the nineteenth century, the global average temperature has risen by approximately 1°C—a trend that is expected to persist for decades to come.

In light of these challenges, it is crucial not only to increase agricultural output but also to maintain the consistency and quality of production. The adoption of innovative technologies and the advancement of sustainable farming practices are essential steps toward achieving these goals (Arora, 2019; IPCC, 2023; Dellal et al., 2025).

Moreover, climate change has detrimental effects on crop yields, with the most significant impacts projected in major cereal-producing regions. By 2050, global maize output is projected to decline by 24%, rice by 11%, and wheat by 3% compared to levels recorded in 2000 (Nishimoto, 2019). These reductions highlight the vulnerability of staple crops to changing environmental conditions and underscore the need for adapting agricultural practices. Maintaining the global supply-demand balance for cereals under these conditions is becoming increasingly challenging. Given the combined challenges posed by climate change and increasing population growth, rapidly enhancing the amount of land under cultivation is not viewed as a viable long-term strategy. As a result, improving the efficiency and productivity of agriculture on existing farmland has become crucial for meeting the food demands of a increasing global population. This necessity underscores the importance of utilizing agricultural inputs more effectively and efficiently—particularly pesticides—and integrating innovative technological approaches into agriculture (Nishimoto, 2019).

2. Crop Protection Products Market

In 2022, global pesticide uses in agriculture totaled 3.70 million metric tons (Mt) of active ingredients, representing a 4% increase from 2021 and a 13% rise over past decade. Compared to 1990, overall pesticide usage has been doubled (FAO, 2025). From 1990 to 2022, the intensity of pesticide application increased at varying rates: usage per hectare rose by 94%, per unit of crop production value by 5%, and per capita use by 35% (FAO, 2025). In 2022, the total volume of pesticide exports declined by 1% compared to 2021, reaching approximately 6.9 million metric tons of formulated products. However, the export value increased by 13%, amounting to 48.8 billion USD (FAO, 2025). That same year, Asia was the leading region for pesticide exports, with 3.5 million metric tons and $21.7 billion, while exports to other regions totaled 2.3 million metric tons and $15.3 billion, respectively (FAO, 2025). In the Americas, agricultural pesticide use rose from 1.71 million metric tons in 2021 to 1.89 million metric tons in 2022, representing a 10% increase (FAO, 2025). In Africa, the majority of pesticide imports originate from outside the region, whereas most pesticides exported from the continent remain within it (FAO, 2025). In Europe, pesticide use has declined by 5% since 1990 and by 7% over the past decade (FAO, 2025). In Oceania, data from four island countries and territories indicate that biopesticides accounted for a median share of 44% of total insecticide use (FAO, 2025).

The crop protection chemicals market is expected to reach $102.4 billion by 2025, with forecasts suggesting further growth to $134.7 billion by 2030. This reflects a compound annual growth rate of 5.63% (Mordor Intelligence, 2025). The sector is experiencing significant transformation, mainly due to changing climate patterns and evolving agricultural practices. Rising temperatures and shifts in rainfall are creating conditions that favor pest outbreaks, allowing pests to expand their geographic range. At the same time, the increasing reliance on intensive and monoculture farming systems amplifies the need for effective pest management, as these systems often provide ideal environments for the spread of pests and diseases.

Technological progress in pesticide application is also reshaping crop protection. Modern methods, such as precision agriculture and integrated pest management, enable a more targeted and efficient use of crop protection products. In 2023, notable advancements were made in seed treatment technologies. For example, companies like Corteva Agriscience® expanded their operations by opening new facilities in regions such as South Africa (Mordor Intelligence, 2025). These developments are complemented by the introduction of new active ingredients and formulations designed to reduce environmental impact and address the persistent issue of pest resistance.

The global crop protection industry continues to face challenges posed by increasing pest-induced crop losses, which threaten global food security. For instance, plant-parasitic nematodes alone are responsible for an estimated annual economic loss of approximately 125 billion USD worldwide, underscoring the critical need for effective crop protection solutions (Mordor Intelligence, 2025). In Europe, diseases such as Septoria leaf blotch (caused by *Septoria tritici* STB) result in annual yield losses of approximately $ 263 million for producers in the United Kingdom, underscoring the significant economic impact of fungal diseases on crop production (Mordor Intelligence, 2025). Additionally, climate change is creating more favorable conditions for particular pest species, posing serious threats to crop production. For example, the 2020 locust infestation severely affected nine countries in East Africa, eleven countries in North Africa and the Middle East, and three in South Asia, resulting in an estimated loss of 8.5 billion USD (Mordor Intelligence, 2025).

There is a clear shift in the industry toward sustainable agricultural practices and integrated pest management approaches. In 2023, major industry players developed new products focused on environmental stewardship and resistance management. For example, Syngenta strengthened its position in the tomato market by launching Orondis Ultra—a significant innovation for controlling late blight (caused by *Phytophthora infestans*). The industry is also responding to regional challenges, such as the threat posed by pests that cause annual losses of between 8.3 and 20.6 million metric tons in twelve major maize-producing countries in Africa, by promoting the development of more effective and sustainable pest management solutions (Mordor Intelligence, 2025).

3. Global Crop Protection Products Markets

3.1. Global Pesticide Usages

In recent years, unexpected outbreaks of pests and diseases driven by climate change have led to a marked increase in pesticide use. According to FAO data from 2022, global agricultural pesticide consumption was reported at 3,690,935 metric tons of active ingredients (FAO, 2025). This substantial level of consumption clearly illustrates the widespread dependence on crop protection products in contemporary plant production.

The data presented in Table 1 demonstrate a consistent rise in pesticide usages between 2018 and 2022. Total pesticide consumption increased from 3,399,053 metric tons of active ingredients in 2018 to 3,690,935 metric tons in 2022. This upward trend suggests a growing reliance on pesticides in agriculture, likely due to efforts to increase productivity. However, evaluating total pesticide usage alone does not provide a sufficient basis for comparison, given the differences in crop production capacity, cultivated area, and population size among countries. Thus, pesticide usage per unit of cultivated land and capita provide more meaningful indicators for comparing countries.

Pesticide use per hectare has also increased over the years. In 2018, this figure was calculated at 2.18 kg/ha, rising to 2.37 kg/ha in 2022 (Table 1). This indicator reflects the average amount of pesticide applied per hectare of agricultural land. It can vary significantly between countries due to differences in agricultural intensity, cropping patterns, and pesticide use practices. Notably, regions with high productivity targets and intense pest pressure are expected to exhibit higher rates of pest infestations (FAO, 2025).

In contrast, per capita pesticide usage remained relatively stable between 2018 and 2022. The value was 0.45 kg per person in 2018 and 0.46 kg per person in 2022 (Table 1). The near constancy of this indicator suggests that, alongside general population growth, the impact of pesticide usage per person has remained parallel. This metric plays a crucial role in evaluating the potential impacts of pesticides on public health and the environment. In countries where per capita pesticide use is high, issues related to pesticide exposure and associated health risks are likely to be more prominent (FAO, 2025).

Overall, the increase in both total pesticide use and application rates per unit of farmland indicates a growing dependence on chemical crop protection, likely driven by efforts to boost yields. However, this trend raises environmental concerns that require careful consideration. The need for sustainable agricultural practices becomes more urgent as pesticide usage rises. Consequently, the escalating use of pesticides emerges as a key issue, requiring ongoing attention from policymakers and environmental managers.

Table 1. Trends in Global Pesticide Use, 2018–2022 (FAO, 2025)

	2018	**2019**	**2020**	**2021**	**2022**
Total Pesticide Usage (tons/ha)	3.399.053	3.481.210	3.447.761	3.566.064	3.690.935
Pesticide Usage in Cultivated Area (kg/ha)	2,18	2,25	2,22	2,29	2,37
Pesticide Usage (kg/person)	0,45	0,46	0,44	0,45	0,46

3.2. Pesticide Use Quantities by Country

Pesticides are now a vital part of modern crop production, primarily used to enhance yields and mitigate losses from pests and diseases. In recent years, increased reliance on pesticides has become a key aspect of crop management in both developed and developing countries. Brazil, the United States, and Indonesia are among the top consumers, together accounting for a large share of global pesticide use (FAO, 2025). Data show that Brazil is responsible for 22% of worldwide consumption, using 800,652 metric tons of active ingredients. The United States follows, applying 467,677 metric tons, which makes up 13% of the total. Other countries, including Indonesia, Argentina, and China, also report significant use of pesticides. Türkiye, for example, uses 52,963 metric tons, which is about 1% of the global total. Table 2 shows annual pesticide usage by country and their percentage shares of the global total.

Table 2. Pesticide Use Amounts and Shares by Country in 2022 (FAO, 2025)

Country	**Amount of Pesticide Used (Active Ingredient, Tons)**	**%**
Brazil	800.652	22
USA	467.677	13
Indonesia	294.670	8
Argentina	262.507	7
China	224.717	6
Vietnam	161.908	4
Canada	97.692	3
Russia	97.018	3
Colombia	78.231	2
France	67.875	2
Australia	59.634	2
Nigeria	57.823	2
Spain	56.353	2
Türkiye	52.963	1
Japan	50.320	1
Other Countries	860.895	23
Total	**3.690.935**	**100**

The global distribution of pesticide use is closely linked to countries' crop production capacities, climatic conditions, and agricultural policies. In major agricultural economies such as Brazil and the United States, extensive cultivated areas and ambitious productivity targets contribute to high levels of pesticide consumption. In contrast, countries like Türkiye exhibit lower pesticide usage, primarily due to more limited arable land and different agricultural practices.

3.3. Annual Pesticide Use per Cultivated Area by Country

Evaluating pesticide use not only in terms of total volume but also on a per-hectare basis reveals significant differences in agricultural practices among countries. The amount of pesticides applied per unit of cultivated area provides a more unambiguous indication of a country's agricultural intensity and its reliance on pesticides (FAO, 2025). In some countries, per-hectare pesticide use is considerably higher than the global average. For instance, in Qatar, 35.1 kg of pesticides are applied per hectare, while in Andorra, this figure is 23.6 kg/ha. Similarly, countries such as Costa Rica, Hong Kong (China), Colombia, and Israel also report notably high per-hectare pesticide use. In Türkiye, the figure stands at 2.2 kg/ha, just below the global average of 2.4 kg/ha (Table 3).

Table 3. Country-Level Pesticide Usage per Hectare (kg/ha) (FAO, 2025)

Country	**Pesticide Use in Cultivated Area (kg/ha)**
Qatar	35,1
Andorra	23,6
Costa Rica	17,5
Hong Kong	16,7
Colombia	16,0
Israel	14,7
Vietnam	13,9
Taiwan	13,5
Republic of Korea	13,0
Brazil	12,6
Japan	11,6
Malta	11,6
Chile	9,9
New Zealand	8,7
Türkiye	2,2
World Average	**2,4**

Table 3 shows that pesticide use in crop production is concentrated in certain countries. For instance, Qatar, Andorra, and Costa Rica have per-hectare usage rates well above the global average. In contrast, Türkiye's per-hectare application is slightly below the world average, suggesting a modest reliance on pesticides in its agriculture.

3.4. Country-Level Annual Per Capita Pesticide Usage

Another important metric for assessing pesticide use is the amount applied per capita. This indicator illustrates the relationship between a country's population and its total pesticide consumption, offering insight into potential exposure levels (FAO, 2025). Analysis reveals that Argentina ranks first globally, with 4.57 kg per person. Other South American countries, such as Uruguay, Brazil, and Paraguay, also report high per capita values. Similarly, countries such as Canada, Australia, and Moldova have usage rates that are well above the global average. In Türkiye, the per capita use of pesticides is 0.62 kg, slightly higher than the global average of 0.5 kg per person (Table 4).

Table 4. Country-Level Annual Pesticide Usage (kg/person) (FAO, 2025)

Country	**Annual Pesticide Use per Person (kg/person)**
Argentina	4,57
Uruguay	4,35
Brazil	3,72
Paraguay	3,7
Canada	2,54
Australia	2,28
Moldova	2,13
Costa Rica	1,76
Vietnam	1,65
Bolivia	1,57
Colombia	1,51
USA	1,38
Spain	1,18
Indonesia	1,07
Türkiye	0,62
World Average	**0,5**

Table 4 shows that per capita pesticide use is considerably higher than the global average, particularly in South American countries. In contrast, Türkiye's per capita pesticide usage is slightly above the world average, indicating a moderate level of pesticide exposure at the population level in Türkiye.

3.5. Annual Pesticide Use by Type and Country

Pesticide usage should be analyzed not only in terms of total quantities but also by type. The distribution of insecticides, herbicides, fungicides-bactericides, plant growth regulators, rodenticides, and other pesticide groups across countries highlights the diversity of crop production strategies and plant protection approaches (FAO, 2025).

For example, in Brazil, herbicide use accounts for a significant portion of total pesticide consumption, with 492,445 metric tons of active ingredients applied. The use of insecticides and fungicides/bactericides is also notably high. In the United States, herbicides account for the largest share of total pesticide use. Indonesia stands out for its high consumption of insecticides, whereas China exhibits a more balanced distribution among different types of pesticides. In Türkiye, the most commonly used pesticide groups are fungicides-bactericides and insecticides (Table 5).

Table 5. Country-Level Pesticide Usage by Type (Tons) (FAO, 2025)

Country	Insecticide	Herbicide	Fungicide–Bactericide	Plant Growth Regulator	Rodenticide	Other Pesticides	Total (Tons)	%
Brazil	127.282	492.445	159.744	8.237		12.944	800.652	22
USA	7.798	405.497	15.075			39.307	467.677	13
Indonesia	171.414	65.318	41.331	6.827	9.779		294.670	8
Argentina	3.354	249.796	4.404	98		4.854	262.507	7
China	63.747	97.663	61.712	2.479	47	10.112	235.760	6
Vietnam	81.664	33.660	45.570	947	68		161.908	4
Canada	4.816	75.280	12.474		10	5.112	97.692	3
Russia	14.274	43.158	35.552	425	326	3.283	97.018	3
Colombia	27.846	29.950	19.858	560	17		78.231	2
France	6.665	29.913	27.933	2.064		1.299	67.875	2
Australia	11.060	44.581	3.672			321	59.634	2
Nigeria	15.872	18.530	19.318	3.281	688	132	57.823	2
Spain	11.034	12.186	32.094	240		723	56.277	2
Türkiye	13.413	13.320	19.096		283	6.851	52.963	1
Japan	14.746	14.003	21.097	472	2		50.320	1
Other Countries							**860.895**	**23**
Total							**3.690.935**	**100**

A comparison between the past decade and the 1990s reveals substantial increases in global pesticide applications: herbicide use has risen by 121%, fungicides and bactericides by 54%, and insecticides by 48%. Over the same period, the distribution among pesticide categories has also shifted. In 2022, global pesticide consumption reached 3.70 million metric tons of active ingredients, marking a 4% rise from 2021 and a 13% increase over the past decade. Additionally, pesticide use has doubled since 1990 (Mordor Intelligence, 2025).

3.6. Annual Insecticide Use by Country

In some countries, the use of insecticides constitutes a significant proportion of total pesticide consumption. Indonesia ranks first in this category, with 171,414 metric tons of active ingredients applied, followed by Brazil with 127,282 metric tons. Other countries such as Vietnam, China, and Colombia also report high levels of insecticide use. In Türkiye, insecticide consumption amounts to 13,413 metric tons of active ingredients, representing a relatively small share of the global total (Table 6).

Table 6. Country-Level Insecticide Usage (Tons) (FAO, 2025)

Country	**Insecticide Usage (Tons)**	**%**
Indonesia	171.414	22,1
Brazil	127.282	16,4
Vietnam	81.664	10,5
China	63.747	8,2
Colombia	27.846	3,6
Pakistan	26.702	3,4
India	20.619	2,7
Germany	17.254	2,2
Nigeria	15.872	2,0
Japan	14.746	1,9
Russia	14.274	1,8
Türkiye	13.413	1,7
Australia	11.060	1,4
Spain	11.034	1,4
Italy	9.817	1,3
Other Countries	148.884	19,2
Total	**775.628**	**100,0**

This table presents the leading countries in insecticide use, along with their respective shares of the global total. The high levels of insecticide consumption observed in Asian and South American countries reflect the significant emphasis placed on pest control in these regions. Türkiye ranks in the middle range for insecticide use, accounting for approximately 1.7% of the global total.

Cypermethrin is classified as a pyrethroid insecticide—a group of synthetic compounds that possess insecticidal activity comparable to that of natural pyrethrins found in chrysanthemum flowers. This compound is widely used for the effective control of various pests, including aphids, leafhoppers, whiteflies, and other insects with larval stages. Cypermethrin acts by interfering with the nervous system of insects, resulting in paralysis and, ultimately, the death of the affected organism. In 2022, the unit price of cypermethrin was reported to be $21,080 per metric ton (Mordor Intelligence, 2025).

Similarly, malathion is an insecticide classified within the organophosphate chemical group. Its mode of action is based on the inhibition of the enzyme acetylcholinesterase, which is essential for nerve function in insects. This property makes malathion particularly effective in controlling pests affecting crops. In 2022, its price was reported to be $12,500 per metric ton (Mordor Intelligence, 2025).

3.7. Annual Herbicide Usage by Country

Herbicides are among the most widely used groups of pesticides in crop production due to their effectiveness in controlling weeds. Globally, herbicide usage varies significantly depending on the size of countries' cultivated areas and their agricultural policies (FAO, 2025). Brazil is the world's leading herbicide user, applying 492,445 metric tons of active ingredients and accounting for 25% of the global total. The United States follows with 405,497 metric tons, representing a 21% share. Other countries, such as Argentina, China, and Canada, also report substantial herbicide consumption. In Türkiye, annual herbicide use amounts to 13,320 metric tons of active ingredients, representing approximately 1% of the global total (Table 7).

Table 7. Country-Level Herbicide Use in 2022 (Tons) (FAO, 2025)

Country	Herbicide Usage (Tons)	%
Brazil	492.445	25
USA	405.497	21
Argentina	249.796	13
China	97.663	5
Canada	75.280	4
Indonesia	65.318	3
Australia	44.581	2
Russia	43.158	2
Vietnam	33.660	2
Colombia	29.950	2
France	29.913	2
Nigeria	18.530	1
Germany	16.812	1
Malaysia	16.036	1
Türkiye	13.320	1
Other Countries	315.029	16
Total	**1.946.989**	**100**

This table highlights the leading countries in herbicide usage, along with their respective shares globally. In particular, herbicide consumption in countries such as Brazil, the United States, and Argentina is closely tied to extensive agricultural areas and ambitious productivity targets. In contrast, Türkiye's herbicide use is below the world average, accounting for only 1% of the global total.

The increasing adaptability of weeds, their rapid reproductive capacity, and the development of herbicide resistance have contributed to the annual rise in herbicide use. When comparing the past decade to the 1990s, global herbicide consumption has increased by 121%. During this period, the share of herbicides in total pesticide use rose from 40% to 50% (FAO, 2025). Notably, glyphosate-resistant *Amaranthus palmeri* S. Watson (ITIS) and *Amaranthus rudis* Sauer, as well as species of the genus *Kochia* resistant to acetolactate synthase inhibitor herbicides, compete with cultivated crops and cause significant yield losses (Mordor Intelligence, 2025).

The active ingredient atrazine is a widely used herbicide for controlling both broadleaf and grassy weeds—such as *Echinocloa*, *Elusine* spp., and

Amaranthus viridis—in crops like maize and rice. In 2022, the price of atrazine was reported to be $13,800 per metric ton. The active ingredient glyphosate was priced at 1,100 USD per metric ton in 2022 (Mordor Intelligence, 2025). According to the FAO (2025), India is the world's largest importer of technical-grade atrazine, while China is the leading exporter of this product. The FAO (2025) report also emphasizes the widespread global use of glyphosate and its increasing market share.

3.8. Annual Fungicide-Bactericide Usage by Country

Fungicides and bactericides are widely used in crop production to control plant pathogens. Globally, the usage of these pesticide groups varies according to factors such as climate conditions, plant diversity, and disease pressure in different countries (FAO, 2025). Brazil is the world's leading user of fungicides and bactericides, applying 159,744 metric tons of active ingredients and accounting for 20% of the global total. Asian countries such as China, Vietnam, and Indonesia also report high levels of usage. In Türkiye, annual fungicide use amounts to 19,096 metric tons of active ingredients, representing 2% of the global total (Table 8).

Table 8. Country-Level Fungicide and Bactericide Use in 2022 (Tons) (FAO, 2025)

Country	Fungicide-Bactericide Usage (Tons)	%
Brazil	159.744	20
China	61.712	8
Vietnam	45.570	6
Indonesia	41.331	5
Russia	35.552	4
Spain	32.094	4
Italy	29.183	4
France	27.933	4
Japan	21.097	3
Colombia	19.858	2
Nigeria	19.318	2
Türkiye	19.096	2
USA	15.075	2
South Africa	14.101	2
Mexico	13.240	2
Other Countries	241.959	30
Total	**796.864**	**100**

Table 8 highlights the countries with the highest levels of fungicide-bactericide usage, detailing their respective contributions to global consumption. The extensive application of these pesticide categories in Brazil and various Asian countries emphasizes the critical role of both preventive and curative disease management strategies in areas facing substantial disease pressure. Türkiye's usage, in this regard, is comparable to the global average.

A comparison of recent data with figures from the 1990s reveals a 54% rise in worldwide fungicide and bactericide use (FAO, 2025). This upward trend underscores the vital role of fungicides in combating fungal diseases, which continue to pose a significant threat to agricultural productivity. Despite their widespread adoption, fungal pathogens affecting cereals, fruits, vegetables, and ornamental crops are estimated to cause annual economic losses of approximately $ 220 billion (Mordor Intelligence, 2025).

3.9. Annual Plant Growth Regulator Use by Country

Plant growth regulators (PGRs) are chemicals used to affect different aspects of plant growth and development. The use of these substances varies between countries, depending on crop patterns, climate, and farming practices (FAO, 2025). Brazil is the largest user of PGRs, applying 8,237 metric tons of active ingredients and accounting for 16% of global usage. Other countries, such as Indonesia, Algeria, and South Africa, also report significant use of PGRs. In contrast, while China, Germany, and France use smaller amounts, PGRs still make up a notable share of their total pesticide use (Table 9).

Table 9. Country-Level Plant Growth Regulator Use in 2022 (Tons) (FAO, 2025)

Country	Plant Growth Regulator Usage (Tons)	%
Brazil	8.237	16
Indonesia	6.827	13
Algeria	3.753	7
South Africa	3.563	7
Nigeria	3.281	6
United Kingdom	2.705	5
China	2.479	5
Germany	2.419	5
France	2.064	4
Other Countries	16.365	32
Total	**51.694**	**100**

Table 9 lists the some countries using plant growth regulators and shows their shares of global usage. Widespread adoption in countries such as Brazil and Indonesia underscores the importance of strategies that focus on enhancing both productivity and product quality in agriculture.

3.10. Annual Rodenticide Use by Country

Rodenticides are an important group of pesticides used to control rodent populations in agriculture. Their use is every day in cereal fields and storage facilities, where they help reduce post-harvest losses and protect stored products. The amount of rodenticide use varies according to countries' agricultural production structures, climatic conditions, and the density of rodent populations (FAO, 2025).

Table 10. Country-Level Rodenticide Usage (Tons) (FAO, 2025)

Country	Amount of Rodenticide Used (Tons)	%
Indonesia	9.779	54,9
Saudi Arabia	2.069	11,6
Malaysia	1.158	6,5
Nigeria	688	3,9
Kuwait	514	2,9
Libya	375	2,1
Russia	326	1,8
Zambia	310	1,7
Morocco	295	1,7
Türkiye	283	1,6
Bosnia and Herzegovina	251	1,4
Cambodia	248	1,4
Haiti	191	1,1
Namibia	177	1,0
Papua New Guinea	71	0,4
Seychelles	70	0,4
Vietnam	68	0,4
Bolivia	57	0,3
Benin	57	0,3
Thailand	51	0,3
China	47	0,3
Total	**17.807**	**100**

According to 2022 data, Indonesia is the world's leading user of rodenticides, applying 9,779 metric tons of active ingredients. Other countries, such as Saudi Arabia, Malaysia, and Nigeria, also report high levels of rodenticide use. In Türkiye, annual rodenticide consumption amounts to 283 metric tons of active ingredients, placing the country in the mid-range globally (Table 10).

able 10 highlights the leading countries in rodenticide use, along with their respective shares of the global total. The high levels of rodenticide consumption in countries such as Indonesia and Saudi Arabia reflect the significant emphasis placed on rodent control in these regions. Türkiye, meanwhile, ranks at a moderate level in rodenticide use and is considered a notable user within its region.

4. Trends in Pesticide Application Site on the Plant

Foliar Applications

In the crop protection products market, foliar application methods accounted for approximately 45% of the sector's total value in 2024. This large share is attributed to the widespread use of foliar spraying, which offers fast and effective control of pests and diseases. The broad leaf surface allows for more even pesticide coverage and better targeting of pests. Additionally, foliar applications are compatible with various pesticide types and allow precise treatment of specific plant parts, making them ideal for large-scale farming. As a result, foliar application remains the preferred choice for many producers (Mordor Intelligence, 2025).

Soil Application

Soil application techniques are expected to show the highest growth potential in the crop protection chemicals market from 2024 to 2029, with a projected CAGR of about 5%. The growing use of soil applications is primarily due to a greater awareness of the importance of soil health for crop productivity and the long-term benefits of controlling soil-borne pests and diseases. Technological advances have improved the efficiency and environmental sustainability of soil application products and methods. The frequent occurrence of soil-borne diseases and the need for effective pest management are also driving the growth of these practices (Mordor Intelligence, 2025).

Other Application Methods

Other important methods for applying crop protection chemicals include chemigation, seed treatment, and fumigation. Chemigation, particularly when combined with modern irrigation systems, enables precise and efficient pesticide distribution, enhancing pest control and promoting water conservation. Seed treatment protects plants during their most vulnerable early stages, reducing the total amount of pesticides needed. While fumigation holds a smaller market share, it remains essential for pest control in storage facilities and for managing soil-borne pests. Altogether, these methods contribute to a comprehensive approach to pest management in crop production (Mordor Intelligence, 2025).

5. Trends in Pesticide Usage by Crops

Pesticide Use in Cereals

In 2024, pesticide use in the cereals and pulses group accounted for approximately 45% of the global pesticide market, making it the largest segment. This is due to the widespread cultivation of crops like wheat, barley, oats, rye, maize, rice, millet, and triticale worldwide. Rising pest and disease pressure in these crops continues to boost their market share. Farmers rely on fungicides, insecticides, and herbicides to achieve high yields and maintain quality (Mordor Intelligence, 2025).

Pesticide Use in Pulses and Oilseeds

Pesticide use in the pulses and oilseeds category is expected to see the fastest growth among crop groups from 2024 to 2029, with an estimated CAGR of about 5%. This is mainly due to increased pest pressure and crop losses in primary pulse and oilseed-producing areas. The growing global demand for protein-rich pulses and vegetable oils is also driving more investment in crop protection for these products. Producers are adopting recent pest management methods and using more pesticides to protect these valuable crops. The expansion of cultivated land and the need for higher productivity further support the strong growth in the pulses and oilseeds sector (Mordor Intelligence, 2025).

Pesticide Use in Other Crop Groups

Other important crop groups in the market include fruits and vegetables, commercial crops, and ornamentals. The fruits and vegetables segment plays a critical role in protecting high-value horticultural crops from a variety of pests and diseases. Commercial crops encompass economically significant products such as tobacco, coffee, tea, rubber, cocoa, and cotton, each requiring specialized solutions for their unique pest and disease challenges. Although ornamentals segment holds a smaller market share, it is important for maintaining the health of golf courses, lawns, landscapes, ornamental greenhouses, and nurseries. Moreover, the use of organic pesticides is increasing in these segments, offering more sustainable alternatives (Mordor Intelligence, 2025).

6. Recent Developments in Global Crop Protection Industry

6.1. Corporate Mergers and Shifts in Market Leadership

Although the crop protection industry has experienced significant growth in recent years, it has also undergone a substantial consolidation among leading agrochemical companies. In 1990, more than ten major agrochemical firms operated in the United States and Europe. By 2009, this number had decreased to six, resulting from mergers and acquisitions (M&A) involving companies such as Syngenta, Bayer, BASF, Dow Chemical, DuPont, and Monsanto. These six companies underwent restructuring due to various factors, including weak financial performance, rising R&D costs driven by increased regulatory requirements for registering new agrochemicals, shareholder expectations for new growth strategies, and other diverse influences. In the subsequent period, further mergers and acquisitions reduced the number of major players to four: Bayer, Corteva (formerly DowDuPont), BASF, and ChemChina (formerly Syngenta). In 2017, FMC Corporation acquired a significant portion of DuPont's crop protection business and R&D assets, which were divested during the merger of DuPont and Dow Chemical. As a result, the current crop protection market is dominated by five leading companies: Bayer, Corteva, BASF, Syngenta, and FMC Corporation (Table 11) (MacDonald, 2017; Nishimoto, 2019).

Table 11. The World's "Big Six" Agricultural Chemical Companies (2015 Data) (MacDonald, 2017)

Company	Country	2015 Seed and Biotechnology Sales (million USD)	2015Agricultural ChemicalSales(million USD)	Proposed Merger Partner
BASF®	Germany	Low*	6.211	Absent
Bayer®	Germany	819	9.548	Monsanto®
Dow Chemical®	USA	1.409	4.977	DuPont®
DuPont®	USA	6.785	3.013	Dow Chemical®
Monsanto®	USA	10.243	4.758	Bayer®
Syngenta®	Switzerland	2.838	10.005	ChemChina®

*Note: BASF® does not report seed sales separately; this item is included under the "other" category.

6.2. Agrochemicals

The current state of R&D in the agrochemical sector and the factors driving company mergers can be viewed from several angles. Most importantly, agrochemicals make a significant contribution to crop production. Studies indicate that unsuitable usage of agrochemicals, yields of staple crops such as rice, wheat, maize, and soybeans could decline by 20% to 50% (Oerke, 2006). Conversely, studies indicate that agrochemicals can prevent 60–70% of potential yield losses, highlighting their crucial role in maintaining current crop production levels. The evidence suggests that the ongoing viability of agricultural output is closely tied to the effective use of these chemicals. Additionally, more agricultural yields can be obtained through more efficient and practical agrochemical applications, underlining the ongoing need for technological innovation in this field (MacDonald, 2017; Nishimoto, 2019).

6.3. Research and Development and Costs

While agrochemicals have played a crucial role in enhancing crop productivity, developing new products in this field has become increasingly complex and resource-intensive. Bringing a new agrochemical to market now typically takes over ten years and requires R&D investments ranging from USD 100 million to USD 350 million. This comprehensive process involves identifying candidate molecules, conducting safety and efficacy studies, developing formulations, analyzing results in detail, and seeking regulatory approval. The chance of successfully commercializing a new agrochemical is very low, with only one out of about 160,000 compounds reaching the market.

As a result, agrochemical companies must allocate 7–10% of annual sales to R&D. Both the costs and time required for development have continued to rise. For instance, in 1995, developing a new product cost$152 million and took 8.3 years.

In contrast, during the 2010–2014 period, these figures increased to $ 286 million and 11.3 years, respectively (McDougall, 2017; Nishimoto, 2019). Notably, plant production costs escalate rapidly in the later stages of development, including environmental and toxicological studies, as well as field trials. The main drivers of this increase include growing demand for safer agrochemicals and increasingly stringent regulatory requirements. Indeed, the number of new agrochemicals peaked in the 1990s and has declined since the 2000s (MacDonald, 2017; Nishimoto, 2019).

6.4. Dosage and Safety in Application

The application rates of agrochemical active ingredients per hectare have dropped significantly over time. In the 1950s, average rates were about 2,400 grams per hectare (g/ha) for herbicides, 1,700 g/ha for insecticides, and 1,200 g/ha for fungicides. By the 2000s, these figures had fallen to between 60 and 180 g/ha (McDougall, 2015; McDougall, 2017; Nishimoto, 2019). This reduction reflects a notable improvement in the biological effectiveness of recently used active ingredients.

Significant progress has also been made in safety. In the 1960s, almost half of the agrochemicals used in Japan were classified as “Designated Poisonous Substances” or “Poisonous Substances.” By 2014, this proportion had dropped sharply, and now, about 90% of agrochemicals are considered “Ordinary Substances” (Nishimoto, 2019). These developments clearly show ongoing efforts to create products that are safer for both people and the environment.

6.5. Biological Pesticides Market

In recent years, there has been a notable increase in research aimed at developing biological pesticides that offer reduced environmental impact and improved safety. Biological pesticides are characterized by the use of active ingredients derived from living organisms. Narrowly, this group comprises only living agents, such as beneficial insects; however, a broader definition extends to include microorganisms and their derivatives. For this study, the broader definition of biological pesticides is employed.

An analysis of newly introduced products over the years reveals a decline in the number of chemical pesticides since the 1990s. In contrast, biological pesticides have shown an upward trend since the 1980s, with an average of ten new biological pesticides entering the market each year since the 1990s (Nishimoto, 2019). However, biological pesticides still hold a limited market share; in 2016, they comprised just 5.6% of the global crop protection market.

6.6. Legislative Frameworks and Sector Dynamics

One of the most significant factors shaping industry trends is the regulatory framework governing the registration processes of agrochemical products. In particular, regulations concerning agrochemicals have become increasingly stringent each year in both the United States and the European Union. In the European Union (EU), the shift in 2011 from risk-based to hazard-based safety and environmental assessment criteria, along with the implementation of "cut-off" criteria, has led to a reduction in the number of registered chemical products. Environmental concerns, such as Colony Collapse Disorder, have triggered strong political responses, resulting in the complete ban of three types of neonicotinoid insecticides for outdoor usage.

Additionally, in the EU, the evaluation of technical and formulated products at multiple administrative levels (EU, regional, and national) in two or three stages significantly prolongs the registration process. Likewise, in Brazil, where the crop protection market is experiencing rapid growth, the vertically structured administrative system requires extensive and intricate review processes. Additionally, Brazil is planning to implement "cut-off" criteria. In Japan, amendments to the Agricultural Chemicals Regulation Act in 2018 introduced a re-evaluation system and new risk assessment frameworks for terrestrial and aquatic organisms as well as for worker exposure.

These developments indicate that the requirements for both initial and re-registration of agrochemicals are becoming increasingly stringent each year, resulting in continuously rising development costs. This situation is considered one of the main drivers behind the recent surge in mergers and acquisitions among major agrochemical companies in the United States and Europe (Nishimoto, 2019).

7. Emerging Technologies

Since the 1990s, leading agrochemical companies in the United States and Europe have expanded their portfolios to include the seed sector alongside traditional chemical pesticide activities. More recently, major firms have not only diversified into new business areas but also accelerated R&D by developing innovative technologies to boost crop productivity. In addition to classical pesticide development methods—such as chemical synthesis, biological evaluation, formulation, and safety and environmental impact analyses—the integration of genetically modified organism (GMO) seed technology has enabled new applications that speed up research and broaden business opportunities. These advances have allowed agrochemical companies to use their technological strengths more effectively, creating new avenues for growth (Nishimoto, 2019).

In recent years, genome editing has become one of the most advanced "genetic recombination" technologies used in developing genetically modified plant varieties. The CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeat) system, introduced in 2013, is notable for its expected wide application in fields such as basic research, medicine, and agriculture. In crop production, CRISPR/Cas9 enables more precise genetic modifications than traditional methods, significantly reducing the time required for plant breeding (Aljabali et al., 2024). Major agrochemical companies have incorporated this technology into their R&D processes, reporting various research findings (Nishimoto, 2019).

Currently, the regulatory status of genome-edited plants—specifically, whether they should be evaluated under the same framework as existing GMO products—remains a subject of intense debate in Japan, the United States, and Europe. Each nation is currently evaluating whether genome-edited products should be regulated in the same manner as conventional GMOs, and it is expected that reaching an international consensus on this matter will require considerable time. Moreover, even after regulatory uncertainties are resolved, public acceptance of genome-edited products will continue to be a significant topic of discussion. The public inevitably expects rational and scientifically grounded explanations regarding these products. If these challenges are addressed effectively, genome editing technology is expected to make substantial contributions to agricultural productivity by enabling the development of high-performance seeds in a significantly more productive compared to conventional breeding methods.

Factors causing yield loss in crop production can be categorized into two main groups: “biotic stress” factors, such as pathogens, harmful animal organisms, and weeds, and “abiotic (environmental/physiogenic) stress" factors, including temperature, frost, drought, and salinity. Traditional chemical pesticides have primarily targeted biotic stress factors, thereby helping to maintain target yield levels. However, analyses of maximum historical crop yields indicate that environmental stress factors have a more decisive impact on yield than biotic stressors. Therefore, effective management of environmental stress is critical for achieving and even increasing target yield levels. In this context, major agrochemical companies are conducting intensive research and development (R&D) activities to develop new agrochemical compounds that can effectively mitigate environmental stress. It has also been reported that some existing agrochemicals may be repurposed for this objective. Furthermore, research continues to develop GMO seed varieties with enhanced resistance to environmental stress. Managing crop stress presents greater opportunities for yield enhancement than conventional agrochemicals targeting biotic stress, and substantial technological advancements are anticipated in this area in the future (Nishimoto, 2019).

A fundamental factor contributing to the rise of smart agriculture has been the transformation of crop management strategies. Whereas yield improvements were once sought through the application of inputs in uncertain amounts, contemporary sustainable agricultural practices now emphasize the reduction or optimization of chemical usage and the enhancement of labor efficiency. Three key technological developments—genome editing, crop stress management, and precision agriculture—have been explored. Much like the transformative influence of GMO products on agriculture in the 1990s, these emerging technologies hold the potential to reshape the sector in the years ahead. There is considerable optimism that these innovations will lead to increased yields and superior crop quality. Looking forward, it will be essential for future researchers and practitioners to integrate these new technologies effectively while ensuring that conventional agrochemicals remain compatible with evolving agricultural systems.

8. Market Trends in Genetically Modified Organisms (GMO)

In recent years, leading agrochemical companies have invested significantly not only in traditional crop protection products but also in genetically modified (GMO) seeds, with this sector now accounting for a substantial portion of their total sales. In 2024, the global market for genetically modified foods reached a value of 133.4 billion USD, and it is projected to grow at a compound annual growth rate (CAGR) of approximately 8.25%, reaching 272.28 billion USD by 2033 (Business Research Insights, 2025). One notable example of strategic growth in this area is Bayer CropScience Limited's acquisition of Monsanto India Limited for $ 63 billion in September 2019. This acquisition was aimed at expanding Bayer's customer base and market share in India. Following the transaction, Bayer retained the Monsanto brand names within its portfolio to continue leveraging their market recognition and brand equity. Established in 1949, Monsanto India Limited® had previously operated as a subsidiary of the American biotechnology giant Monsanto® (Business Research Insights, 2025).

The genetically modified foods market is categorized according to both product type and application. Key product types include vegetables, field crops, animal-derived products, and fruits, while application areas encompass herbicide tolerance, insect resistance, and stacked traits. Leading companies in the industry pursue organic growth through product approvals, new product introductions, and intellectual property initiatives, as well as inorganic growth via mergers, acquisitions, strategic alliances, and collaborative ventures. These approaches enable companies to expand their customer portfolios and revenues; as global demand for genetically modified foods continues to rise, industry stakeholders are expected to benefit from significant growth opportunities in the near future. Leading companies in the global GMO foods market include Syngenta, Dow Chemical Company, Groupe Limagrain, Bayer CropScience, Sakata Japan, DuPont, Monsanto, KWS SAAT SE, and BASF GmbH (Business Research Insights, 2025).

8.1. Corporate Strategies in the Genetically Modified Seed Sector

Since the 1990s, leading agrochemical companies have adopted an integrated marketing strategy that includes the sale of genetically modified

(GMO) seeds alongside agrochemical products. In this context, major firms have acquired seed companies, developed GMO seed products, and significantly expanded their activities in the field of crop protection. According to the 2016 Phillips McDougall report (McDougall, 2017), the seed market share within the total sales of the former six major companies was reported as 21% for Syngenta®, 14% for Bayer®, 70% for DuPont®, 25% for Dow Chemical®, and 75% for Monsanto® (McDougall, 2017; Nishimoto, 2019).

In parallel with this increase in seed sales, a marked rise in R&D expenditures has also been reported. In 2002, the combined R&D spending of these six major companies was approximately $1.2 billion; by 2008, this figure had reached $2.2 billion, approaching the level of R&D investment in chemical pesticides. By 2014, total R&D expenditures had risen to 4 billion USD, about 1.5 times the amount spent on chemical pesticides (Nishimoto, 2019). This trend illustrates the strategic approach of major agrochemical companies to expand the seed segment further. Moreover, the increase in R&D spending on seed products is directly related to the rise in the number of new seeds introduced to the market (Nishimoto, 2019).

9. Sustainability and Food Safety in the Crop Protection Sector

Pesticides have been instrumental in improving crop yields, and the continued sustainability of present production levels is now widely regarded as being closely linked to their practical application. Agrochemical companies continuously develop and introduce new products to support crop production; however, increasingly stringent global regulatory requirements are making the process of developing new products ever more complex and challenging. In contrast, the introduction of genetically modified crops in the 1990s contributed to achieving target yield levels and has come to represent a substantial share of major agrochemical companies' sales. In the medium and long term, innovative approaches such as the development of new plant varieties through genome editing techniques and the establishment of sustainable agricultural systems via smart farming are expected to open new horizons for the sector.

With the world population projected to exceed 10 billion by 2050 (FAO, 2024), increasing yield per unit area has become an unavoidable necessity due to the limited availability of arable land. Furthermore, addressing global food security challenges is becoming increasingly complex in the face of the adverse effects of climate change. In this context, the primary responsibility of professionals in the crop protection industry is to contribute to achieving crop yield targets and solving global sustainability challenges by developing new products and technologies. These efforts are essential for achieving the United Nations Sustainable Development Goals, particularly ‘Zero Hunger’ (Goal 2), as well as all related objectives.

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CHAPTER 8

PHYTOTOXIC EFFECT OF *VITEX AGNUS-CASTUS* L.

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INTRODUCTION

Weeds can cause yield losses of up to 100% in agricultural production areas if left uncontrolled. Although various control methods are used worldwide and in our country, chemical control remains the most commonly applied method. Herbicides are widely preferred because, in addition to their rapid and decisive effects, they require less labor (Hussain et al., 2014; Serim et al., 2015). However, it is well known that herbicides used in weed control have adverse impacts on environmental integrity. Safe food production in relation to agricultural practices and agrochemicals is of great importance. Although synthetic herbicides are useful tools, their irresponsible use may lead to yield losses, environmental contamination, and the development of herbicide-resistant weed species (Koul and Walia, 2009; Hazrati et al., 2018).

The concept of allelopathy has emerged as a remarkable alternative solution within agricultural ecosystems. Allelopathy is defined as the process by which one organism affects another, whether beneficial or harmful, through the production of allelochemicals (Rice, 2012). In particular, allelopathic substances used to suppress the germination of weed seeds offer a promising and environmentally friendly alternative for weed control (Horuz and Üremiş, 2024).

Plants belonging to the Verbenaceae family have been traditionally used in medicine in various countries. To date, the Verbenaceae family comprises approximately 3,000 species, including shrubs, small trees, and, rarely, herbaceous plants distributed in tropical and subtropical regions (Rahmatullah et al., 2011; Özderin, 2021). The genus *Vitex* is one of the largest genera of the Verbenaceae family, comprising a total of 217 species, mostly distributed in the Mediterranean region, Central Asia, and Southern Europe. Among them, the best-known species are *Vitex agnus-castus* L., *Vitex trifolia* L., *Vitex cymosa*, *Vitex rotundifolia*, and *Vitex negundo* L. (Altunay and Taştan, 2023). In Turkey, only two species are naturally found: *Vitex agnus-castus* and *Vitex negundo* L. (Dutta and Dutta, 1964). *Vitex agnus-castus* L. is a deciduous shrub or small tree with a rounded crown, reaching 3–6 m in height. It has palmate leaves with 5–7 leaflets; the upper surface is green, while the lower surface is densely white-tomentose. The plant bears slender, elongated inflorescences of blue-purple flowers, 8–10 cm in length (Stojkovic et al., 2011; Niroumand et al., 2018).

Previous studies have reported that *Vitex agnus-castus* exhibits a wide range of biological activities, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, spasmolytic, antiviral, and antifungal effects (Peng et al., 2008; Gökbulut et al., 2010; Yilar et al., 2016).

This study aimed to investigate the allelopathic effects of methanol extracts obtained from different parts (root, leaf, and seed) of *Vitex agnus-castus* on the germination and seedling growth of selected test plants (garden cress, alfalfa, wheat, and hollyhock).

MATERIALS AND METHODS

Plant Material

Vitex agnus-castus L. is naturally distributed in various regions of Turkey (Figure 1). The plant material used in this study was collected from the province of Antalya during its flowering stage in the vegetation period. The collected plants were dried in the shade under laboratory conditions and then ground into powder using an electric grinder.

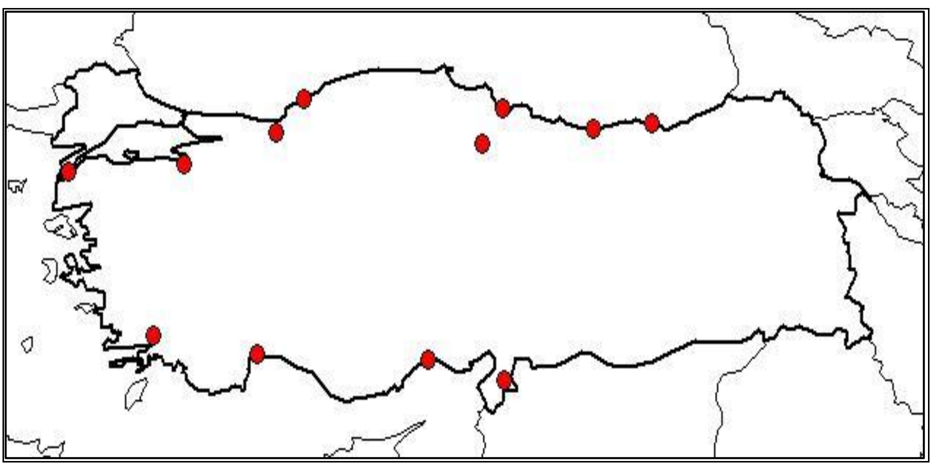

Figure 1. *Vitex agnus-castus* bitkisinin Türkiye'deki yayılış gösterdiği alanlar (Anonim, 2025)

Preparation of Plant Extract

A total of 200 g of the dried and ground plant sample was placed into a 2-liter Erlenmeyer flask, and methanol was added until the plant material was completely covered. The mixture was shaken on an orbital shaker at 120 rpm for 24 hours, then filtered through filter paper to remove solid residues. Subsequently, the filtrate was centrifuged at 5000 rpm for 15 minutes to ensure complete separation of solid particles. The resulting extract was dissolved in DMSO to prepare solutions at concentrations of 100, 200, and 400 ppm (Yılar and Bayar, 2023). The extracts were stored at +4 °C until use in the experiments.

Allelopathic Effect of the Plant Extract on Test Plants

The effects of the plant extract on seed germination and seedling growth of the test plants were investigated in 9-cm diameter Petri dishes. Two layers of filter paper were placed in each Petri dish, and seeds of the test plants (25 seeds per dish) were evenly distributed. Different doses of the plant extract (100, 200, and 400 ppm) as well as the control (DMSO + distilled water) were applied by adding 5 mL of solution to each dish. The Petri dishes were sealed with parafilm and incubated at approximately 24 °C for 1–3 weeks. At the end of the incubation period, germination rates and root and shoot lengths were recorded (Yılar and Bayar, 2023). The experiments were carried out with four replications and two independent repeats. The percentage inhibition of the extracts was calculated according to the following formula:

$$I : 100x\left(dc - dt\right)/dc$$

where I is Inhibition rate, dc is the number or length in the control group, and dt is the corresponding value in the treatment group.

Data Analysis

The significance of differences among treatments was determined by analysis of variance (ANOVA), and mean comparisons were performed using Duncan’s multiple range test. Statistical analyses were conducted using the SPSS 15.0 software package.

RESULTS AND DISCUSSION

Methanol extracts obtained from different parts of *Vitex agnus-castus* significantly affected seed germination, root growth, and shoot development of the tested plant species at the $p < 0.05$ significance level. However, these effects varied depending on the extract type, application dose, and test plant (Table 1, Figure 2). Differences in effect were also observed depending on the plant part and increasing concentration of the methanol extract. For wheat seed germination, the flower methanol extract at 400 ppm (Ç400) showed the strongest inhibitory effect (100%), followed by the leaf extract at 400 ppm (Y400) with 43% inhibition and the root extract at 400 ppm (T400) with 25% inhibition. The negative effects of the extracts on wheat root and shoot growth were consistent with their effects on seed germination (Table 1; Figures 2,3, 7,8,9).

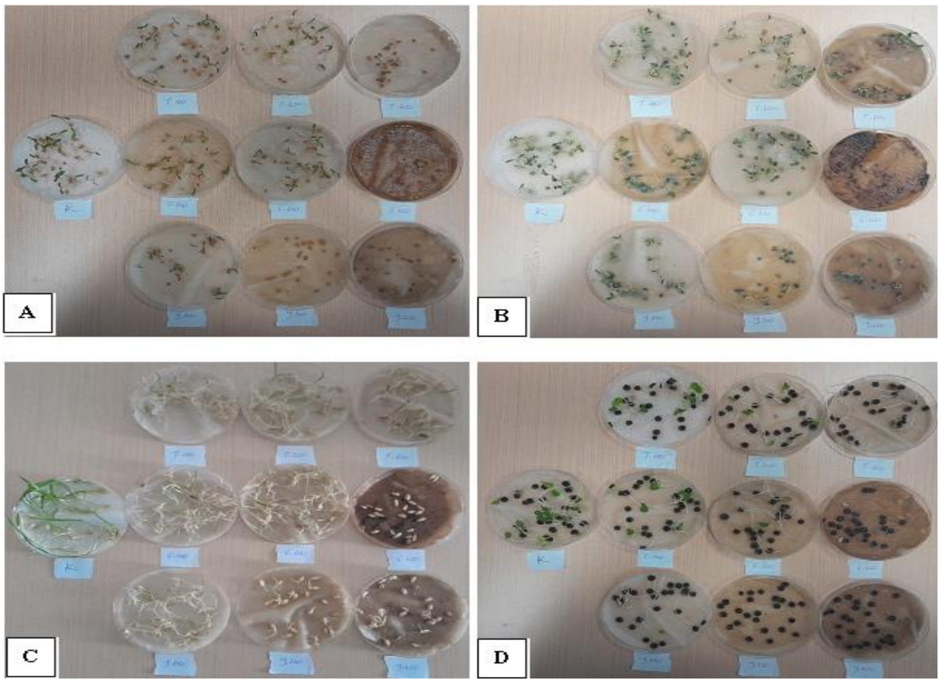

Figure 2. Effect of plant extracts on seed germination of test plants (A: Cress, B: Clover, C: Wheat, D: Hollyhock)

Seed germination of *Lepidium sativum* (garden cress) was completely inhibited by the leaf and flower methanol extracts at a concentration of 400 ppm. In contrast, the seed methanol extract inhibited seed germination by 66% compared to the control. The leaf and flower methanol extracts also completely suppressed seedling growth (Table 1, Figure 2, Figure 6, Figure 7). The seed methanol extract inhibited root growth of cress by 62.02%–85.61% compared to the control (Figure 2). Similarly, shoot growth of cress was inhibited by 62.0%–75.68% by the seed extract (Figure 8, Figure 9). For *Medicago sativa* (alfalfa), the flower methanol extract exhibited the strongest inhibitory effect on seed germination and seedling growth, followed by the leaf and seed extracts

(Table1, Figure 2, Figure 5, Figure 7-9). Among the test plants, *Alcea rosea* (hollyhock) was identified as the most sensitive species. The leaf methanol extract at 200 ppm, and the flower methanol extract at 400 ppm, completely inhibited seed germination as well as root and shoot development (Table 1, Figure 2, Figure 4, Figure 7-9).

Figure 3. Effect of plant extracts on wheat seedling growth

Figure 4. Effect of plant extracts on rose marshmallow seedling growth

Figure 5. Effect of plant extracts on alfalfa seedling growth

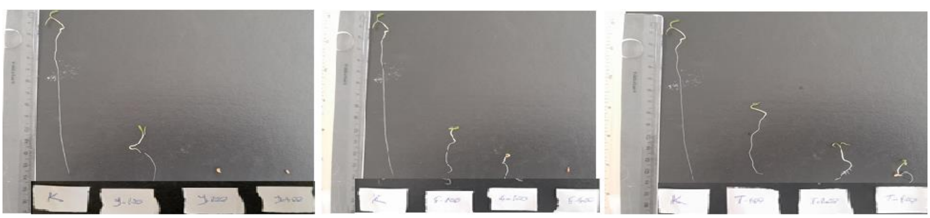

Figure 6. Effect of plant extracts on cress seedling growth

Table 1. The effect of methanol extract obtained from *Vitex agnus-castus* plant parts on test plants

Test plants		Extracts Doses(ppm)									
		Control	T_{100}	T_{200}	T_{400}	Y_{100}	Y_{200}	Y_{400}	$Ç_{100}$	$Ç_{200}$	$Ç_{400}$
***Triticum aestivum* L.**	**%GR**	$100.0^{a*}\pm0.0$	$91.0^{c}\pm1.0$	$90.0^{c}\pm1.1$	$75.0^{e}\pm1.0$	$97.0^{b}\pm1.1$	$81.0^{d}\pm1.1$	$57.0^{f}\pm1.1$	$91.0^{c}\pm1.1$	$89.0^{c}\pm1.1$	$0.0^{g}\pm0.0$
	RL(mm)	$13.89^{a}\pm0.4$	$7.66^{b}\pm1.2$	$4.45^{dc}\pm0.6$	$2.76^{de}\pm0.2$	$5.27^{c}\pm0.5$	$2.49^{e}\pm0.5$	$1.26^{fe}\pm0.2$	$4.65^{dc}\pm0.6$	$4.41^{dc}\pm0.7$	$0.0^{f}\pm0.0$
	SL(mm)	$8.97^{a}\pm0.6$	$7.36^{ba}\pm1.1$	$6.20^{cba}\pm1.3$	$4.49^{cb}\pm0.8$	$4.55^{cb}\pm0.9$	$4.15^{cb}\pm1.3$	$2.80^{dc}\pm0.8$	$8.05^{ba}\pm2.1$	$6.85^{ba}\pm1.5$	$0.0^{d}\pm0.0$
***Lepidium sativum* L.**	**%GR**	$100.0^{a}\pm0.0$	$82.0^{a}\pm1.1$	$77.0^{a}\pm1.0$	$34.0^{b}\pm2.0$	$90.0^{a}\pm1.1$	$10.0^{c}\pm2.0$	$0.0^{c}\pm0.0$	$88.0^{a}\pm2.8$	$74.0^{a}\pm2.4$	$0.0^{c}\pm0.0$
	RL(mm)	$8.69^{a}\pm1.3$	$3.30^{b}\pm1.01$	$3.29^{b}\pm2.3$	$1.25^{b}\pm0.12$	$2.22^{b}\pm0.5$	$1.04^{b}\pm0.2$	$0.00^{b}\pm0.0$	$3.24^{b}\pm1.17$	$3.06^{b}\pm0.7$	$0.0^{b}\pm0.0$
	SL(mm)	$3.29^{a}\pm0.52$	$1.25^{b}\pm0.08$	$1.19^{b}\pm0.16$	$0.8^{b}\pm0.14$	$1.15^{b}\pm0.10$	$1.04^{b}\pm0.28$	$0.0^{c}\pm0.0$	$1.05^{b}\pm0.05$	$1.0^{b}\pm0.05$	$0.0^{c}\pm0.0$
***Medicago sativa* L.**	**%GR**	$100.0^{a}\pm0.0$	$77.0^{c}\pm1.0$	$77.0^{c}\pm1.0$	$60.0^{e}\pm0.0$	$85.0^{b}\pm1.0$	$34.0^{f}\pm2.0$	$26.0^{g}\pm2.0$	$72.0^{d}\pm0.0$	$59.0^{e}\pm1.0$	$0.0^{h}\pm0.0$
	RL(mm)	$3.01^{a}\pm0.35$	$2.08^{b}\pm0.15$	$1.49^{c}\pm0.12$	$0.71^{ed}\pm0.09$	$1.3^{c}\pm0.12$	$0.56^{ed}\pm0.13$	$0.32f^{e}\pm0.11$	$1.03^{dc}\pm0.22$	$0.75^{ed}\pm0.07$	$0.0^{f}\pm0.0$
	SL(mm)	$2.55^{a}\pm0.54$	$1.65^{b}\pm0.15$	$1.35^{cb}\pm0.05$	$0.73^{dc}\pm0.10$	$1.1^{dcb}\pm0.10$	$0.47^{ed}\pm0.02$	$0.70^{dc}\pm0.20$	$1.01^{dcb}\pm0.18$	$0.8^{dc}\pm0.03$	$0.0^{e}\pm0.0$
***Alcea rosea* L.**	**%GR**	$90.0^{a}\pm5.7$	$62.0^{b}\pm3.8$	$40.0^{c}\pm3.2$	$36.0^{c}\pm2.3$	$40.0^{c}\pm3.2$	$0.0^{d}\pm0.0$	$0.0^{d}\pm0.0$	$41.0^{c}\pm1.9$	$34.0^{c}\pm2.0$	$0.0^{d}\pm0.0$
	RL(mm)	$5.08^{a}\pm0.10$	$2.94^{cb}\pm0.41$	$2.83^{cb}\pm0.30$	$2.52^{c}\pm0.0$	$2.82^{cb}\pm0.21$	$0.0^{d}\pm0.0$	$0.0^{d}\pm0.0$	$3.50^{b}\pm0.17$	$3.10^{cb}\pm0.42$	$0.0^{d}\pm0.0$
	SL(mm)	$1.78^{a}\pm0.13$	$0.68^{b}\pm0.03$	$0.34^{dc}\pm0.02$	$0.25^{d}\pm0.03$	$0.25^{d}\pm0.02$	$0.0^{e}\pm0.0$	$0.0^{e}\pm0.0$	$0.68^{b}\pm0.01$	$0.45^{c}\pm$	$0.0^{e}\pm0.0$

* Means with the same letter in the same row were not significantly different by ANOVA (a = 0.05)[RL:Root length, SL: shoot length, GR: germination,T: Seed , Y:leaf , Ç: Flower].

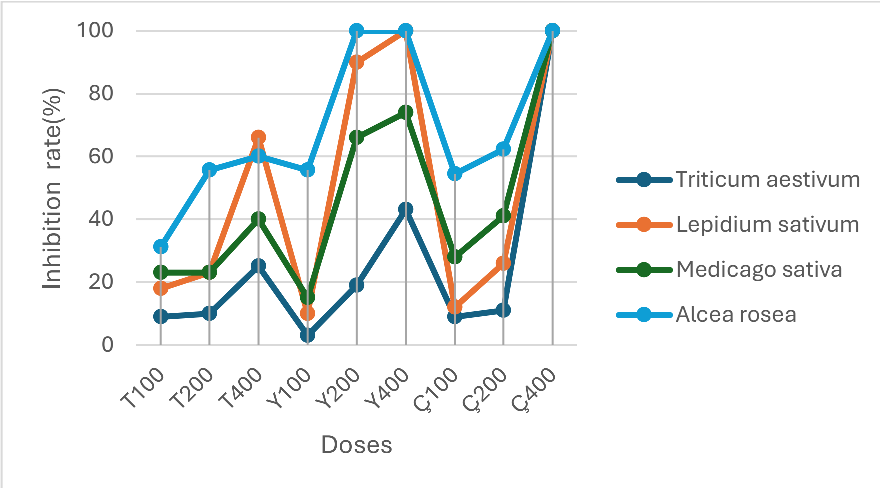

Figure 7. Inhibition rate(%) on seed germination of test plant of plant extracts

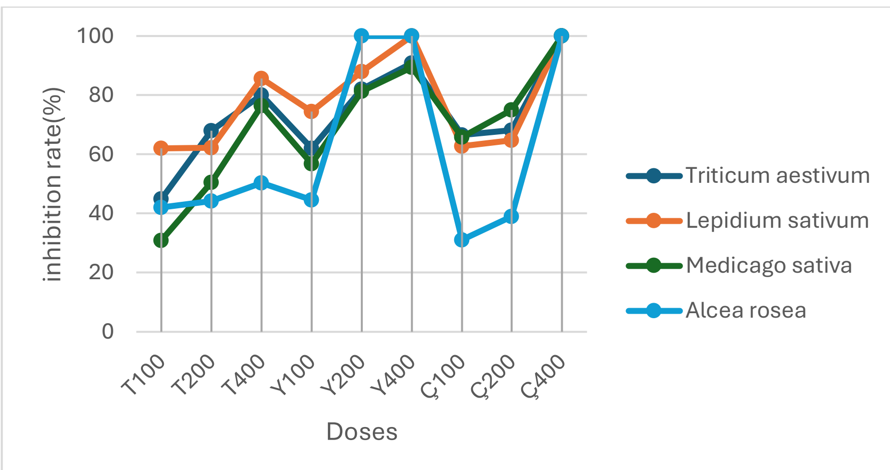

Figure 8. Inhibition rate(%) on root growth of test plant of plant extracts

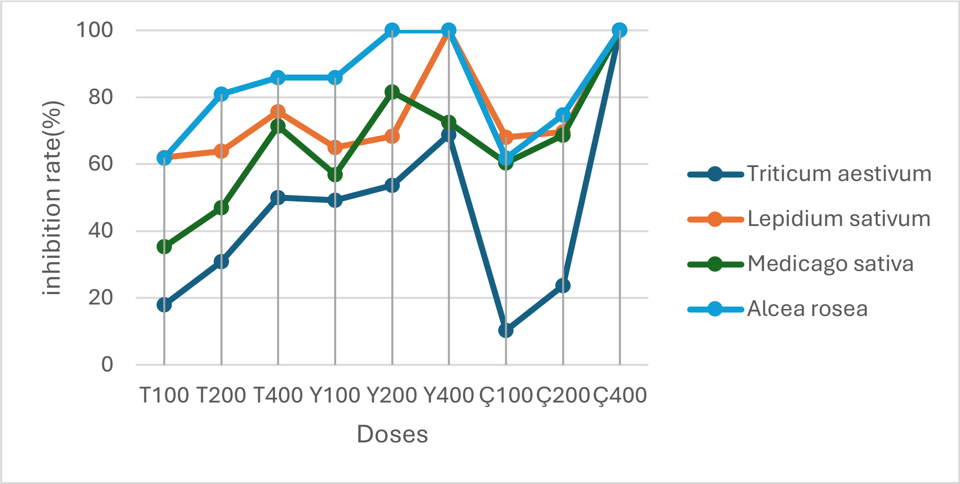

Figure 9. Inhibition rate(%) on shoot growth of test plant of plant extracts

Allelopathy is an environmentally advantageous approach that can control weeds both within and outside agricultural areas, increase crop yield, reduce the use of synthetic chemicals in agriculture, and help restore beneficial microorganisms (Adeola, 2012; Naeem et al., 2018). Allelopathy is defined as the phenomenon in which an organism affects other living organisms through allelochemicals it produces and releases into the environment (Rice, 2012). Studies are being conducted on the potential use of these allelochemicals from plants for weed management. Moreover, many researchers have reported that these compounds may have the potential to replace synthetic herbicides (Jabran, 2017). The use of medicinal and aromatic plants as natural herbicides has also been documented due to their allelochemical contents (Cruz-Ortega et al., 2007; Mahdavikia and Saharkhiz, 2015).

Vitex agnus-castus L. (Verbenaceae) is a perennial plant known for its extensive use in traditional medicine, and it also attracts attention due to its environmental interactions. The ability of this species to inhibit or stimulate the growth and development of surrounding plants indicates its allelopathic activity (Zeqiri et al., 2022; Di Simone et al., 2025). Phenolic compounds, flavonoids, and essential oils reported in *V. agnus-castus* are thought to play a role in such effects. The main active constituents of *Vitex* have been reported as flavonoids, including casticin, apigenin, vitexin, isovitexin, luteolin, orientin, isoorientin, santin, and 6″-caffeoylisoorientin. Furthermore, different parts of this plant have been reported to exhibit anti-inflammatory, antimicrobial, antifungal, antioxidant, and anticancer activities (Girman et al., 2003; Souto et al., 2020; Altunay and Taştan, 2023). *Vitex* species are well known not only for their industrial, pharmacological, and toxicological uses but also for their essential oil content and phytotoxic activity (Haghighi and Saharkhiz, 2021). In this study, the allelopathic activity of the medicinal plant *Vitex agnus-castus* was also demonstrated.

CONCLUSION

Under laboratory conditions, methanol extracts obtained from different parts of *Vitex agnus-castus* were tested for their allelopathic effects on plants. According to the results, the plant exhibited strong allelopathic activity. This pronounced effect is associated with the presence of secondary metabolites in the plant. Indeed, due to these secondary metabolites, numerous studies have been conducted on the potential use of this plant in the treatment of various diseases. Based on the findings of this study, we believe that investigating the allelopathic potential of this plant may also offer new perspectives for both ecological and agricultural systems.

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CHAPTER 9

SOME AQUATIC ORGANISMS USED FOR MICROPLASTIC STUDIES IN TÜRKİYE'S INLAND WATERS

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1. INTRODUCTION

Plastics have an important place in our daily lives due to their abilities such as being light and floating in water, polymer-containing, lipophilic and insulating, resistant to chemicals, easy to process and shape, recyclable and more durable with various additives (Aydın et al., 2023). For this reason, in order to respond to the increasing population demand, plastic production increased continuously from 1.5 million tons in the 1950s to 413.8 million tons in 2023 (PlasticEurope, 2024).

Depending on this production amount, plastics are a worrying environmental problem for countries (Bakhshaee et al., 2025; Megha et al., 2025). Microplastics (MPs), which are formed by the gradual breakdown of waste plastics (effects such as sunlight, waves, currents and oxidation) that can be found in many places in the natural environment (Atici et al., 2020; Atici et al., 2022; Gündoğdu et al., 2023), into smaller pieces (plastics smaller than 5 mm) (Arthur et al., 2009), can pass into aquatic environments and be swallowed unnoticed by many aquatic organisms (Atici et al., 2021; Atici, 2022; Gedik & Atasaral, 2022; Mülayim et al., 2022; Atıcı, 2024; Atici, 2025; Zilifli et al., 2025). When the first records are examined, it was reported that MP was ingested by fishes in the North Atlantic Ocean in 1970 (Carpenter et al., 1972).

Today, studies on MPs have accelerated, and researchers have reported that MP contamination occurs in many water sources, including fish and many other aquatic organisms. In this study, it is aimed to give the MP characteristics of some aquatic organisms used in MP studies in the inland waters of Türkiye and as a result to evaluate the MP studies in the inland waters of Türkiye in general on aquatic organisms.

2. AQUATIC ORGANISMS

2. 1. Fishes

MP studies conducted on fishes in various inland waters including lakes, dam lakes, ponds, and streams in Türkiye are presented in Table 1. The most frequently studied water sources for MP contamination in fish were streams (9), lakes (6), and dam lakes (5), respectively. The most frequently studied fish species was *Cyprinus carpio* (8 publications), followed by members of the *Carassius* (5 publications) genus.

The most common MP colors and types in fish were blue color and fiber type. The determined polymer types were polyethylene (PE), high density polyethylene (HDPE), polyester (PES), polypropylene (PP), polyethylene terephthalate (PET), polyvinyl chloride (PVC), polyamide (PA), polystyrene (PS), polyacrylate (PA), polyphenylene sulfide (PPS), polycyclohexylenedimethylene terephthalate (PCT), polyvinyl acetate (PVA), polychloroprene chlorine (PCP), poliakrilamid (PAM), ethylene-vinyl acetate (EVA), poly(vinyl stearate) (PVS), polyisobutylene (PIB), nitrile butadiene rubber (NBR), acrylic (PAN), ethylene propylene (EPR), polibütilen tereftalat (PBT), ethyl-acrylate (EA), polyacrylic (PACs), polysulfide rubber (PSR), neopren (polikloropren), and cellulose (Table 1).

The highest mean MP value found in the digestive system of fish per individual (ind.) was reported in *Albunur taricihi* (34 ± 13 items/ind.) (Atici et al., 2021), Turkish Salmon (23 ± 8.6 items/ind.) (Atıcı, 2024), *Luciobarbus capito* (12.2 ± 6.7 items/ind.) and *Silurus glanis* (12.5 ± 2.7 items/ind.) (Birge, 2023) species (Table 1).

Table 1. MP characteristics in some fishes reported from different water systems of Türkiye

Water body type	**Species**	**Location**	**Study findings**	**Study authors**
Lake	*A. tarichi*	Van Lake	Mean MP: 34 ± 13 (8-124) items/ind. Dominant MP type and color: Fiber (74%) type and blue (58%) color Polymer types: PE and PP	Atici et al. (2021)
River	*Carassius gibelio*	Mert River (Samsun)	Total MP amount: 234 items (57 fibers, 91 fragments, 80 particles, 6 films) for upper river samples and 241 items (96 fibers, 89 fragments, 51 particles, 5 films) for lower river samples	Çini Barutçu & Polat (2021)

River	*Cyprinus carpio, Mugil cephalus*, *Capoeta umbla, Capoeta trutta, Blicca bjoerkna*, *Atherina mochon, Squalius squalus,* and *Garra rufa*	Erzurum, Erzincan and Bingöl Streams	Dominant MP sizes, type and color: 0-50 to 50-100 µm size, fragment and fiber types, and black color The max MP amounts: 53.0% for *C. carpio* in Erzurum, 29.7% for *C. umbla* and 26.6% for *B. bjoerkna* in Erzincan and 20.7% for *S. squalus* and 18.2% for *B. bjoerkna* in Bingöl Polimer types: PP, PET, PSR, PCP, PVC, PIB, EPR, EA, and PA (polyacrylate)	Atamanalp et al. (2022a)
River	*C. carpio, Squalius cephalus,* and *Alburnus mossulensis*	Karasu River (Erzurum)	Max. MP size: 1001-2000 mm Dominant MP type and color: Fiber type and black color Polymer types: PE, PES, PVS, PET, PP, PS, and cellulose	Atamanalp et al. (2022b)
River	*C. gibelio, C. carpio, Planiliza abu, Anguilla anguilla, Clarias gariepinus,* and *Carassius auratus*	Orontes River (Hatay)	Mean MP: 4.4 ± 2 items/ind. in gills and 5.1 ± 2 items/ind. in gastrointestinal tracts (GITs). Dominant MP size, type and color: < 1000 µm size, fiber type and black color Polimer types: PES (50%), HDPE (10%), PP (8%), and PET (5%)	Kılıç et al. (2022)
Dam lake	*C. carpio* and *A. mossulensis*	Sürgü Dam Lake (Malatya)	MP range: 0-3 (mean 0.41) items/ind. Dominant MP size, type and color: 0.2-1	Turhan (2022)

			mm size, fiber type and transparent color Polymer types: PET and PP	
River	*Alburnus sellal* and parasite, *Ligula intestinalis*	Upper Tigris River	Mean MP: 1.27 ± 1.30 items/fish (57% of 536 *A. sellal* specimens) Mean MP amount: 1.77 ± 1.79 items/parasite (74% of 57 *L. intestinalis* specimens) Dominant MP type and color: Fiber (96.2% for fishes and 81% for parasites) type and black (37% for fishes and 58% for parasites) color Dominant polymer type: Acrylic (PAN) for both species	Aytan et al. (2023)
River	*L. capito* and *S. glanis*	Aras River (Iğdır)	Mean MP of GITs: 12.2 ± 6.7 items for *L. capito* and 12.5 ± 2.7 items for *S. glanis* Mean MP amounts of gills: 8.9 ± 2.6 items for *L. capito* and 9.1 ± 4.2 items for *S. glanis* Dominant MP size: 101-200 μm for both species Dominant MP types: Fiber type for both species (GITs: 64.8% for *L. capito* and 64.8% for *S. glanis,* and gills: 66.3% for *L. capito* and 53.8% for *S. glanis*) Dominant MP colors: Black color for both species	Birge (2023)

			(GITs: 68.0% for *L. capito* and 64.0% for *S. glanis,* and gills: 64.0% for *L. capito* and 61.5% for *S. glanis*) Polymer types: PE (29.5%), PP (21.0%), PA (polyamide, 18.1%), PBT (11.4%), PAM (9.5%), Neopren (Polikloropren, 7.6%), and PIB (2.9%)	
Lake and dam lake	*Scardinius erythrophthalmus, Alburnus spp., C. carpio, C. gibelio, Neogobius fluviatilis, Vimba vimba,* and *Perca fluviatilis*	Uluabat, Manyas, Gökgöl and Gala Lakes, and Alaçatı, Tahtalı, Beydağ, and Karaidemir Dam Lakes	MP size range: 0.10-4.85 mm Dominant MP type and color: Fiber type and blue color	Böyükalan & Yerli (2023)
Lake and river	*C. gibelio*	Susurluk River Basin	Mean MP size: 1.17 ± 0.95 Dominant MP type and color: Fiber (97.4%) type and black (56.6%) color	Kankılıç et al. (2023)
Lake	*Mugil cephalus*	Cernek Lake (Samsun)	Mean MP: 2.87 ± 0.27 items/ind. or 0.014 ± 0.001 items/g Mean MP size: 1522.4 ± 131.2 (132-4850) µm Dominant MP type: Fiber (67.47%) Polymer types: PET (44.5%), PP (34.94%), PE (10.84%), and PA (polyamide, 9.64%)	Terzi (2023a)
Lake	*C. gibelio*	Karaboğaz, Balık, and Liman Lakes	Mean MP: 3.20 ± 0.18 items/ind. or 0.036 ± 0.002 items/g	Terzi (2023b)

		(Kızılırmak Delta)	MP size range: 112-4775 µm MP types: Fiber (78.0%), fragment (13.46%), and film (8.54%) types Polymer types: PET (56.91%), PP (25.20%), PE (9.76%), and PA (polyamide, 8.13%)	
River	*Barbus anatolicus, C. carpio,* and *Capoeta tinca*	Kızılırmak (Nevşehir)	Total MP amount: 76 items for *B. anatolicus*, 192 items for *C. carpio* and 96 items for *C. tinca* Dominant MP type and color: Fiber type and white color Polymer types: PA (polyamide), PP, nylon, PE, and PS	Aras (2024)
Farmed fish (Inland water)	Turkish Salmon (*Oncorhynchus mykiss*)	Purchased from the supermarket (Van)	Mean MP: 23 ± 8.6 (14-31) items/ind. Dominant MP size, type, shape and color: 0.1-0.3 mm (29.6%) size, fiber (% 73.0) type, elongated (64.3%) shape, and blue/black (42.6%) colors	Atıcı (2024)
River basins	All *Squalius* species	22 different river basins in Türkiye	Mean MP: 0.27 ± 0.19 items/ind. Mean MP size: 1701 ± 1585 (101-4963) µm Dominant MP type and color: Fiber (79.1%) type and black (35.2%) color Dominant polymer types: PE (38.5%) and PET (29.7%)	Gedik et al. (2024)
Dam lake	*Alburnus derjugini* and *Squalius*	Kürtün Dam Lake (Gümüşhane)	Mean MP: 0.334 items/ind. for non-infected fish (NIF),	Minaz et al. (2024)

	orientalis, and parasite *L. intestinalis*		0.233 items/ind. for infected fish (IF), and 0.233 items/ind. for *L. intestinalis* (L) Mean MP size: 3757.6 ± 2451.0 µm for NIF, 1471.1 ± 758.9 µm for IF, and 1824.4 ± 1009.8 µm for L Dominant MP type and color: Fiber (100% for NIF and IF, 85.7% for L) type and black (57.1% for IF and L) and orange (50% for NIF) colors Polymer types: PA (polyamide, 57.1% for IF, 50% for NIF) and PET (28.5% for L)	
Dam lake	*C. carpio, P. fluviatilis, Atherina boyeri,* and *Sander lucioperca*	Yamula Reservoir (Kayseri)	Mean MP: 6 ± 5.9 items/ind. for *C. carpio*, 1.8 ± 1.7 items/ind. for *A. boyeri*, 2 ± 2.8 items/ind. for *S. lucioperca*, and 4.6 ± 6.3 items/ind. for *P. fluviatilis* Dominant MP size, type and color: 0-499 µm size, fiber type, and black color Dominant polymer types: PP (67%), PVC (13%), PE (13%), and HDPE (7%)	Ceylan et al. (2025)
Dam lake	*C. carpio carpio*, *C. carpio*, and *Perca fluviatilis*	Gelingullu Reservoir (Yozgat)	Mean MP: 1.2 ± 1.8 items/ind. for GITs and 0.7 ± 0.9 items/ind. for gills in *C. carpio*, 0.5 ± 0.9 items/ind. for GITs and 0.7 ± 0.8 items/ind. for gills in	Erdoğan (2025)

			C. carpio carpio, and 0.9 ± 1.1 items/ind. for GITs and 0.8 ± 0.7 items/ind. for gills in *P. fluviatilis* Dominant MP size, type and color: 0-100 µm and 100-200 µm sizes, fiber (78.9%) and fragment (21%) types, and blue and black colors Polymer types: PS (50%), PES (25%), and PP (25%)	
Lake	*B. bjoerkna*	Manyas and Uluabat Lakes (Marmara Basin)	Mean MP: 0.03 ± 0.03 items/ind. for Manyas Lake and 0.04 ± 0.02 items/ind. for Ulubat Lake MP type and color: Black fibers and white fragments Polymer types: PA (polyamide) and PET	Kurtul et al. (2025)

2. 2. Invertebrates

The most frequently studied water sources for MP contamination in aquatic invertebrates were reservoirs (6), lakes (4), and streams (3), respectively (Table 2). The most published aquatic invertebrate studies were conducted on individuals of the *Unio* (4 publications), *Dreissena* (3 publications), *Anodonta* (2 publications), and freshwater crayfish genus (3 publications). In addition to bivalves and crayfish, MP contamination has also been reported in freshwater snail species (1 publication).

The most common MP colors detected in aquatic invertebrates were blue, black, white, orange, and transparent, while the most common MP types were fiber, fragment, and film (Table 2). The identified polymer types were PE, PET, PE, PP, PES, PS, PPS, Nylon-6, and cellulose.

The highest mean MP value determined in aquatic invertebrates was reported in *Unio stevenianus* (39.15 ± 16.95 items/ind.) (Atıcı, 2022) and

Astacus leptodactylus (0.89-15.67 items/ind. for GITs, and 0.5-19.67 items/ind. for gills) (Mülayim et al., 2022) (Table 2).

Table 2. MP characteristics in some invertebrates reported from different water systems of Türkiye

Water body type	Species	Location	Study findings	Study authors
River	*U. stevenianus*	Karasu River (Van)	Mean MP: 2.85 ± 1.27 (0.81-6.69) items/g or 39.15 ± 16.95 items/ind. Dominant MP sizes, type and color: < 0.1 mm (44.8%) size, fragment (48.8%) and fiber (47.5%) types, and black (48.8%) color	Atici (2022)
Lake and dam lake	*Anodonta* sp., *Dreissena polymorpha*, *Unio damescensis*	Çıldır Lake, Almus and Kartalkaya Dam Lakes (Ardahan-Kars)	MP range: 0.75-10.0 items/ind. for *Anodonta* sp., 0.16-1.00 items/ind. for *D. polymorpha*, and 0.50-2.50 items/ind. for *U. damescensis* Dominant MP type: Fiber Polymer types: PET (36%)	Gedik & Atasaral (2022)
Lake	*A. leptodactylus*	Durusu Lake (Terkos)	MP range: 0.89-15.67 items/ind. for GITs and 0.5-19.67 items/ind. for gills Dominant MP type and color: Fiber (63.96% for GITs and 83.33% for gills) type, and black (38.58% for GITs and 39.13% for gills) color Polymer types: PP (78%), PPS (11%), and PE (11%)	Mülayim et al. (2022)
Dam lake	*Pontastacus leptodactylus*	Çatalan Dam Lake (Adana)	Mean MP: 2.2 ± 2.2 items/ind. for gills and 11.9 ± 9.7 items/ind. for GITs Dominant MP size, type and color: 1-2.5 mm (35% for gills and 39% for GITs), fiber type (90% for gills and 99% for GITs) type,	Yücel & Kılıç (2022)

			and black (56% for gills and 38% for GITs) color Polymer type: PE (40%), PP (25%), and PES (20%)	
Dam lake	*D. polymorpha*	Beyhan Dam Lake (Elazığ)	MP range: 0.70-1.80 items/ind. or 1.06-3.47 items/g Total MP amount: 52 items Dominant MP size, type and color: 1001–2000 µm size, fiber type and black color Polymer type: PP	Atamanalp et al. (2023)
Dam lake	*Unio mancus*	Atatürk Dam Lake (Adıyaman)	Mean MP: 1.89 ± 0.57 (range 0-9.9) items/ind. or 6.91 ± 2.56 (range 0-50.51) items/100 g Dominant MP size and type: < 100 µm size and fragment type Polymer types: PP (40%), PE (20%), PET (10%), Nylon-6 (10%), and cellulose (10%)	Gündoğdu (2023)
Lake and river	*Anodonta anatina*	Susurluk River Basin	Mean MP size: 6.9 ± 2.2 cm Dominant MP type and color: Fiber (96%) type and black (75.7%) color	Kankılıç et al. (2023)
Dam lake	*A. leptodactylus*	Keban Dam Lake (Elazığ)	Mean MP: 1.95 items/ind. for GITs and 1.30 items/ind. for gills MP size range: 100-500 µm The dominant MP type and color: Fiber (64.1% for GITs and 57.7% for gills) type and black (51.3% for GITs and 57.7% for gills) color Dominant polymer types: PE (30%)	Atamanalp et al. (2025)

Lake and dam lake	*Unio terminalis, Unio delicatus* and *Dreissena polymorpha*	Gölbaşı Lake (Hatay), Çatalan (Adana), Karakaya (Diyarbakır), and Atatürk dam lakes (Adıyaman)	Mean MP: 0.6 ± 0.8 items/ind. or 0.2 ± 0.5 items/g Dominant MP size, type and color: < 250 (40%) µm size, fiber (93%) type, and blue (58%) color Polymer type: PE (40%), PP (47%), and PS (27%)	Yücel et al. (2025)
River	*Potamopyrgus antipodarum*	Kocabaş Stream (Çanakkale)	MP size and type: 2.5 mm size and fibril type	Zilifli et al. (2025)

2. 3. Other Aquatic Organisms

Apart from bivalves, crayfish and snails among aquatic invertebrates, it has been determined that there are also studies on MP contamination in semi-aquatic/amphibian animals such as frogs belonging to the *Pelophylax* genus (5 publications) and *Rana* genus (1 publication), water snakes belonging to the *Natrix* (1 publication) genus and salamanders belonging to the *Neurergus* genus (2 publications) (Table 3).

The most common MP colors detected in semi-aquatic/amphibian organisms were blue, red, and transparent, while the most common MP types were reported as fiber and fragment types. The polymer types determined were PE, PET, PP, PA (polyamide), nylon and PACs, PVC, PVA, EVA, PCT, acrylic, NBR, and nylon (Table 3).

The highest mean MP value determined in semi-aquatic/amphibian organisms was reported in *Pelophylax* spp. (3.17 items/ind.) (Dursun et al., 2023) (Table 3).

Table 3. MP characteristics in some other aquatic organisms reported from various water systems of Türkiye

Water body type	Species	Location	Study findings	Study authors
Pond, puddle, and ditch	*Pelophylax ridibundus* and *Rana macrocnemis*	Fındıklı Puddle, Çamlıhemşin Pond, Salarha and İkizdere Ditchs (Rize)	MP range: 302.62-306.69 items/g Polymer type: PET, nylon, and PACs	Karaoğlu & Gül (2020)
Different regions	*Natrix natrix* and *Natrix tessellata*	Several regions of Anatolia	Mean MP: 1.36-2.38 items/ind. (*N. natrix*) and 1.26-2.43 items/ind. (*N. tessellata*) MP size: 500 µm (*N. natrix*) and 1000-1500 µm (*N. tessellata*) Dominant MP color and type: Blue (52.6%) for *N. natrix* and black (39.4%) for *N. tessellata* and fiber type for both samples (94.7% for *N. natrix* and 87.9% for *N. tessellata*) Polymer type: PET (68.4%), PE (21.1%), PVC (5.3%), and PVA (5.3%) for *N. natrix* and PET (41.2%), PE (29.4%), PVA (11.8%), acrylic (5.9%), PVC (5.9%), (PP) (2.9%), and nylon (2.9%) for *N. tessellata*	Gül et al. (2022)
Different regions	*Pelophylax ridibundus* and *Pelophylax bedriagae*	Several regions of Anatolia	MP range: 0.20-18.93 items/ind. Dominant MP size, type, color and polymer: < 300 µm (90%) size, fiber (92.2%) type, blue (76.1%) color, and PET (70.1%) polymer	Tatlı et al. (2022)
Different sources	*Pelophylax* spp.	Different regions of Türkiye	Mean MP: 3.17 items/ind. MP size range: 66-3770 µm	Dursun et al. (2023)

			Dominant MP type and color: Fiber (97.1%) type and transparent color Dominant polymer type: EVA (37.3%)	
River and spring water	*Neurergus strauchii*	18 different rivers (Eastern Anatolia Region)	Mean MP: 0.53 (range 0-4) items/ind. MP size range: 64-828 µm Dominant MP type and color: Fiber (94%) type and blue (68%) color Polymer types: EVA (61%), PET (20%), PCT (16%), and PE (3%)	Altunışık et al. (2024)
Different habitats	*Neurergus barani* and *Neurergus strauchii*	Malatya and Bitlis	Mean MP: 1.44 ± 0.24 (1-3) items/ind. for *N. barani* and 1.44 ± 0.43 (1-5) items/ind. for *N. strauchii* MP size range: 102.24-1109.72 (mean 523.39 ± 76.38) µm for *N. barani* and 98.16-968.52 (mean 843.56 ± 98.72) µm for *N. strauchii* Dominant MP type and color: Fiber type and blue (47.7%) color Dominant polymer type: PET (63.6%)	Dursun et al. (2024)
Lake	*Pelophylax ridibundus*	Buyukcekmece (Istanbul), Şavşat and Karagol (Artvin)	Mean MP: 0.2 items/ind. for Buyukcekmece and 0.13 items/ind. Şavşat and Karagol populations. MP size: 246-498 µm (mean 357.4 ± 44.31 µm) for both populations MP type: Fiber (67%) and fragment (33%) types for Buyukcekmece Lake, and fiber (100%) type for Şavşat Lake MP color: Blue (67%) and red (33%) colors for Buyukcekmece Lake, and blue (50%) and	Tatlı & Altunışık (2025)

			transparent (50%) colors for Şavşat Lake Polymer types: PET (67%) and EVA (33%) for Buyukcekmece Lake, and PET (100%) for Şavşat Lake	
Different habitats	*Pelophylax bedriagae*, *P. ridibundus*, and *P. caralitanus*	11 different regions	Mean MP: 1.52 (0-4.20) items/ind. MP size: 100-2411.5 (mean 242.30 ± 215.14) µm Dominant MP type and color: Fiber (90.8%) type and blue (68.7%) color Polymer types: EVA (32.5%), PCT (20.8%), PA (polyamide, 12.9), NBR (12.9%), and PET (11.7%)	Tatlı et al. (2025)

Except for the semi-aquatic/amphibious organisms listed in Table 3, a study on MP uptake in the water hyacinth, *Pontederia crassipes* species was also reported by Kılıç & Yücel (2024) in Table 4.

Table 4. MP characteristics in aquatic plant reported from different water systems of Türkiye

Water body type	Species	Location	Study findings	Study authors
River	*P. crassipes*	Orontes River (Hatay)	Mean MP: 42 ± 23 items/ind. Mean MP size: 633 ± 628 µm Dominant MP size, type and color: < 500 µm size, fragment (69%) type and blue (30%) color	Kılıç & Yücel (2024)

3. CONCLUSION

MP studies on aquatic organisms in Türkiye's inland waters show that MPs of various sizes, types and colors are ingested by aquatic organisms, and that the gill tissue and especially the digestive system of the organisms are contaminated with MPs. These studies have shown that other aquatic organisms, such as fish, may be exposed to MP contamination. In the studies, it was seen that blue and black colors and fiber and particle types were the most dominant MP characteristics. In addition to the polymer types such as PE, PET, PE, PP, PES, PS, PPS, Nylon-6, and cellulose, many different polymer types have been identified.

Considering the studies conducted, it can be seen that the consumption of economically important aquatic organisms contaminated with MP for food purposes may pose a risk to human health. In order to prevent MPs from occurring in nature through various ways, it is important to control the sources of plastics that form MPs and not release them into the environment.

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CHAPTER 10

EVALUATION OF POMEGRATANE PEEL EXTRACT ON *A. PLATENSIS* CULTIVATION

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1. INTRODUCTION

Food waste resulting from food processing makes up almost 39% of food waste in European Union countries. A great ratio of this waste is rich in some valuable compounds and is regarded as rich byproducts. Pomegranate is produced widely in Mediterranean and West Asia. Peel:grain:seed ratio is 50:40:10 which makes 60% of the fruit a potential byproduct (Campos et al., 2022). After the production of pomegranate juice is completed within a certain period of time, the pomegranate peel, which is approximately 40% of the whole fruit, remains in nature as a waste. When we look at it, pomegranate peel is an important by-product of the pomegranate fruit processing industry, and at the same time, pomegranate fruit is a rich source of bioactive compounds such as polyphenols and flavonoids (Karataş and Aydoğmuş, 2023). The phenolic content of pomegranate peel is 10 times richer than pulp extract (Li et al., 2006). *Arthrospira platensis,* also called Spirulina, is filamentous trichomial cyanobacterium used in bioremediation, nitrification and carbon dioxide fixation. The microalga is rich in essential amino acids and minerals (Özoğul et al., 2021). This microalga has a commercial importance and can be cultivated, harvested and processed easily (Soni et al., 2019). *Arthrospira platensis* can be cultivated in the climate of Türkiye and can be used in food, pharmaceutical, dye and cosmetics industries (Jung et al., 2019). In this study the pomegranate peel extracts were used in cultivation of *A. platensis* and their effects on growth, biomolecule content and biomass production were evaluated.

2. MATERIALS AND METHODS

Artrospira platensis was obtained from a local provider. Pomegranates were bought from different local markets in Süleymanpaşa, Tekirdağ, Türkiye. The peels of pomegranates were separated from the fruit, chopped into small pieces and dried under infrared lamp for 24 hours.

2.1. Pomegranate peel extracts and antibacterial activity testing

Different solvents (dH_2O, ethyl acetate, methanol, ethanol, and acetone) were used to obtain pomegranate peel extracts in 1:20 (m:V) ratio. The mixture was kept at 45°C for 24 hours and the mixture was filtered; filtrate was added into a new tube and the solvent was evaporated under infrared lamp. The remaining

In order to determine which solvent was the most appropriate for further use in microalgae cultures, antibacterial activities of the extracts obtained with different solvents were tested against *Escherichia coli, Pseudomonas aeruginosa, Salmonella typhimirium, Staphylococcus aureus, Streptococcus mutans,* and *Enterococcus faecalis*. The bacteria were first grown on tryptic soy agar overnight at 37 °C. Agar well diffusion method was used. Bacterial suspensions of 0.5 McFarland were spread on Mueller-Hinton agar plates under aseptic conditions. Wells of 8 mm diameters were formed and 100 µL of extracts were added into the wells. The plates were kept at 37 °C for 24 hours after which the zone diameters around the wells were measured (Çalışkan ve Gürgan Eser, 2024). According to the results of the primary experiments, ethyl acetate was chosen since the antibacterial activity of the ethyl acetate extracts dissolved with distilled water were so lowest (Figure 1 and 2), hence Spirulina microalgae would not be affected from the extracts.

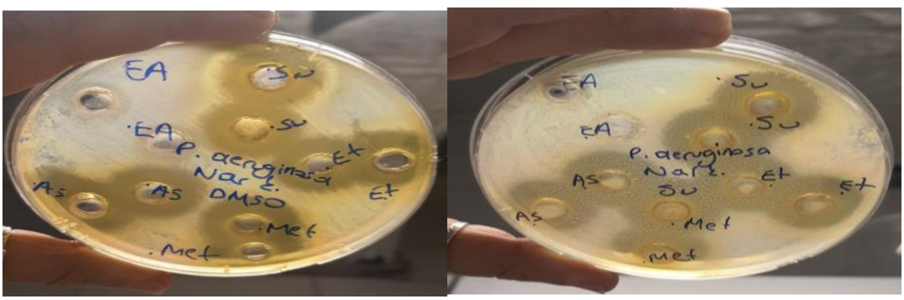

Figure 1. Antibacterial activity test by well diffusion method. Left: Effect of ethyl acetate extract dissolved in DMSO against *P. aeruginosa*. Right: Effect of ethyl acetate extract dissolved in dH_2O against *P. aeruginosa*.

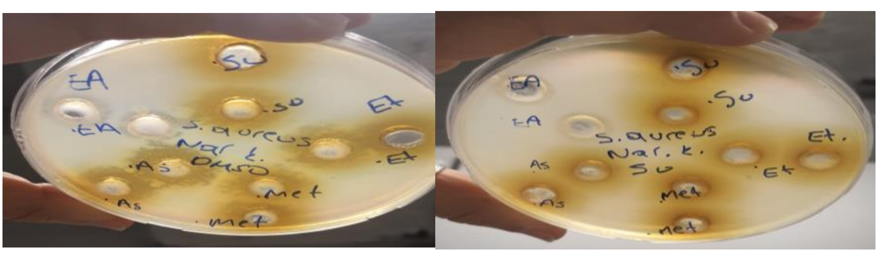

Figure 2. Antibacterial activity test by well diffusion method. Left: Effect of ethyl acetate extract dissolved in DMSO against *S. aureus*. Right: Effect of ethyl acetate extract dissolved in dH_2O against *S. aureus.*

2.2 Experimental set-up

Arthrospira platensis was first activated from -20°C stock on agar plates of Zarrouk's medium and grew at 36°C under 16 h illumination and 8 h dark. The microalgae on agar were passed into liquid Zarrouk's medium and periodically the volume of the culture was culture was increased up to 19 L. From this culture 250 mL of *A. platensis* starter culture was added into the reactor bottle to bring the total volume to 5L with Zarrouk's medium. The ingredients of the Zarrouk's medium are given in Table 1. The experimental set up was prepared with 3 replicates of each group with addition of either 0 (control), 1000, 5000, 10000 or 20000 µL of pomegranate extract obtained with ethyl acetate and dissolved in dH_2O. Cultures were grown for 21 days under white LED light, at 30-35°C ambient temperature with continuous aeration of air pumping into the cultures. The reactor bottles were periodically shifted places to ensure homogeneity about lighting.

Table 1. Zarrouk's medium for *A. platensis*

Ingredient	Amount
$NaHCO_3$ (g)	16.8
$NaNO_3$ (g)	2.5
K_2HPO_4 (g)	0.5
K_2SO_4 (g)	1.0
NaCl (g)	1.0
$MgSO_4.7H_2O$ (g)	0.2
$CaCl_2.2H_2O$ (g)	0.04
$FeSO_4.7H_2O$ (g)	0.01
EDTA(g)	0.08
Micronutrient solution	1mL

Micronutrient solution: H_3BO_3: 2.86 g/L, $MnCl_2.4H_2O$: 1.81 g/L, $ZnSO_4.4H_2O$: 0.222 g/L, Na_2MoO_4: 0.0177 g/L, $CuSO_4.5H_2O$: 0.079 g/L

2.3 Analyses

Optical density of microalgae cultures was measured at 565 nm for biomass concentration, 680 nm for chlorophyll a measurement, and optical density at 750 nm everyday using a spectrophotometer (Shimadzu).

When the pH of the cultures reached 10, the microalgae were harvested using a silk tammy cloth and the microalgae on the cloth were washed 2-3 times to get rid of medium residues. The biomass was dried on glass plates under infrared lamp at 40-42 °C for 12 hours. The dry weight of biomass was weighed.

2.3.1 Chlorophyll analyses

Microalgae biomass was mixed with 80% acetone in 1:30 (m:V) ratio in a microcentrifuge tube and incubated at 4 °C 24 hours, after which the mixture was centrifuged at 10000 rpm for 5 minutes and supernatant was used to determine the chlorophyll contents by measuring optical densities at 645, 665, 650 nm. The following formulae were used to calculate the chlorophyll contents of *A. spirulina*:

$$Chlorophyll\ a\ (mgL^{-1}) = 12.7 \times A_{665} - 2.69 \times A_{645}$$

$$Chlorophyll\ b\ (mgL^{-1}) = 22.9 \times A_{645} - 4.68 \times A_{665}$$

$$Total\ chlorophyll\ \left({}^{mg}/_{L}\right) = (20.2 \times A_{645}) + (8.02 \times A_{663})$$

2.3.2 Carotenoid analysis:

The pellet from chlorophyll measurement was used 1 mL of and 99% ethanol was added onto this pellet, incubated for 40 min at 4°C, mixed well by vortexing and centrifuged at 10000 rpm for 5 min. Optical density of the supernatant was measured at 470 nm and the following formula was used to calculate carotenoid concentration.

$$Total\ carotenoid\ (mg\ L^{-1}) = \big((1000 \times A_{470} - 2.86 \times chla - 129.2 \times chlb)\big) \div 245$$

2.3.3 Phycocyanin analysis:

A mixture of *A. platensis* was prepared in distilled water in a ratio of 1:30 (m:V) and incubated at -18°C for 24 h. After thawing, the mixture was mixed by vortexing and filtered through basic filter paper. The filtrate was used to calculate phycocyanin content by measuring optical densities at 652 and 615 nm and using the following formula:

$$Phycocyanin\ \left({}^{mg}/_{ml}\right) = (A_{615}) - (0.474 \times A_{652}) \div 5.34$$

2.4. Statistical analysis

The data was analyzed using One-way ANOVA at α=0.05 using SPSS 22.0 software. The graphics were drawn using Microsoft Office Excel program.

3. RESULTS AND DISCUSSION

In this study, the effects of different concentrations of pomegranate peel extracts (PPE) obtained by ethyl acetate and dissolved in dH2O into the growth medium on growth, chlorophyll, carotenoid and phycocyanin contents of *A. platensis* microalgae.

3.1 Effects of different PPE concentrations on *Arthrosphra platensis* growth

Spectrophotometric measurements of *A. platensis* were carried on for 22 days until the harvest. Daily measurements at 565 nm, 680 nm and 750 nm were done to determine the biomass concentration, chlorophyll *a* content, and optical density of *A. platensis*, respectively.

The optical density of *A. platensis* cultures with addition of 20.000 µL PPE were higher than the control condition on the final day of the experiment (Figure 3). The lowest was obtained by addition of 5000 µL PPE. Thhe increases in the optical density of the cultures on some days such as Day 9, 12, 16, 19 and 21 were due to the evaporation of culture medium which resulted in higher density of the culture. The following days, decrease in optical density was due to the addition of Zarrouk medium. Hence, it can be suggested that periodic addition of medium without letting the medium evaporate is required.

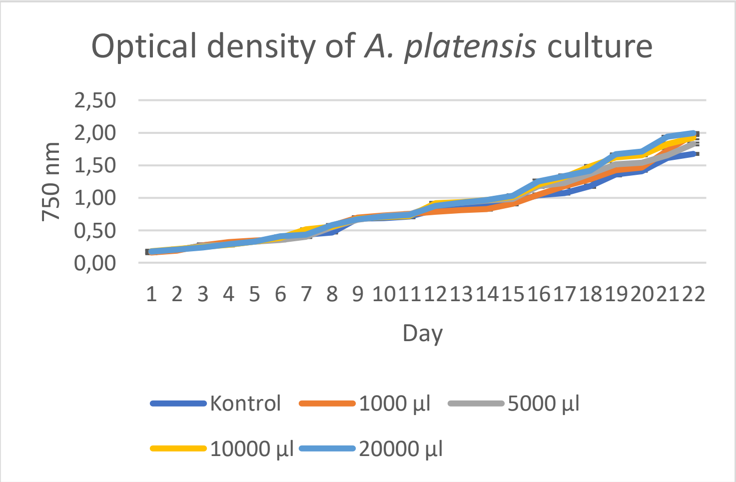

Figure 3. Optical density of *A. platensis* culture with addition of different concentrations of PPE

The biomass of cultures was followed by measuring optical density at 565 nm as shown in Figure 4. The highest biomass was measured on the final day of the experiment in culture with addition of 10000 µL PPE, while control condition had the lowest biomass. Hence, it can be suggested that biomass concentration of *A. platensis* can increase with addition of PPE.

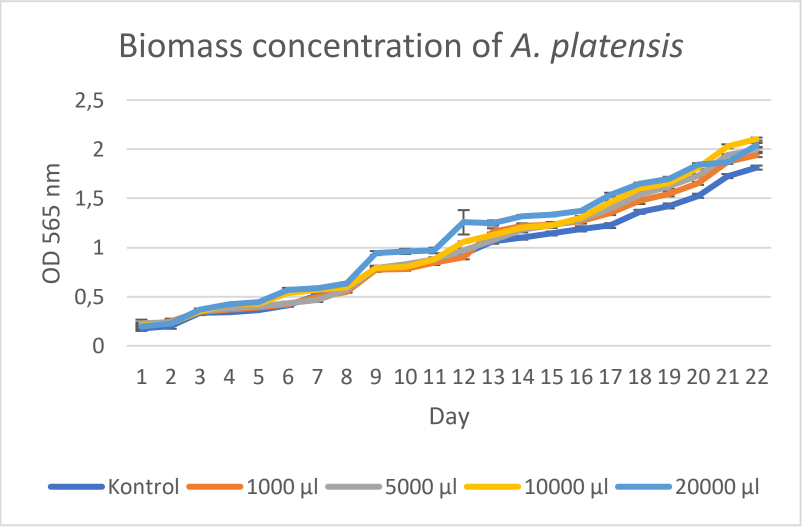

Figure 4. Biomass concentration of *A. platensis* upon addition of different concentrations of PPE

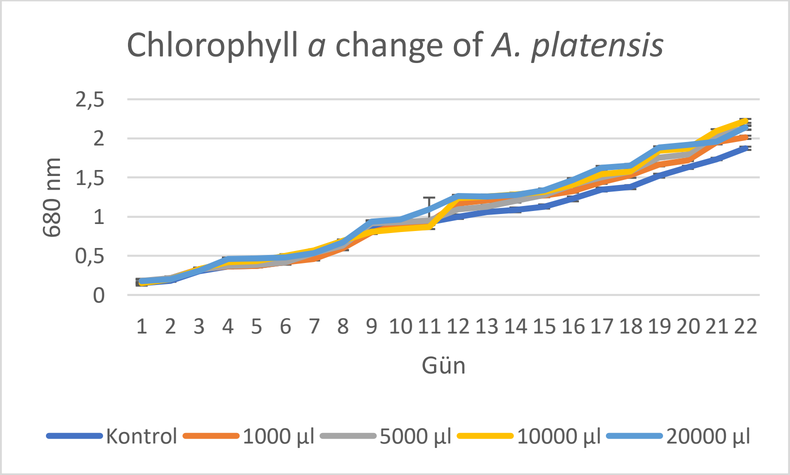

Figure 5. Chlorophyll a change of *A. platensis* upon addition of different concentrations of PPE

The chlorophyll *a* content of *A. platensis* was the highest on the final day of the experiment in the culture with addition of 10000 µL PPE, and the lowest was the control condition (Figure 5). Similarly, chlorophyll *a* was enhanced by addition of PPE.

3.1.1 Effects of PPE on biomass and bioactive compounds of *Arthrospira platensis*

The bioactive compounds of *A. platensis* such as chlorophyll, carotenoid and phycocyanin were evaluated after the harvest and the effects of different concentrations of PPE (pomegranate peel extract) obtained by ethyl acetate were given in Table 2.

Table 2. Effect of pomegranate peel extract (PPE) on biomass and bioactive compounds of *A. platensis*

	Dry weight	Chlorophyll *a* (mg/L)	Chlorophyl l *b* (mg/L)	Total chlorophyll (mg/L)	Carotenoid (mg/L)	Phycocyani n (mg/mL)
Control	1.96±0.08	109.15±7.3	11.62±3.7	118.1±7.4	19.78±3.9	1.28±0.01
1000 µL	1.88±0.08	118.33±0.5	19.28±0.2	128.4±2.7	16.0±0.85	0.69±0.00
5000 µL	2.1±0.007	105.58±5.2	13.58±3.0	114± 5.71	18.1±3.34	0.99 ±0.01
10000 µL	2.84±0.14	93.46±6.39	11.8±2.34	102±4.2	19.6±3.02	1.03 ±0.13
20000 µL	2.52±0.01	105.31±5.2	10.16±1.7	112.7±6.0	20.8±0.07	0.7 ± 0.12
P value	0.000	0.046	0.082	0.037	0.504	0.002

The lowest biomass of *A. platensis* cultures measured after the harvest was obtained in the reactors with 1000 µL PPE, while the highest was obtained by 10 times higher PPE addition. The silk tammy cloth used for the harvesting and filtering can have some spirulina biomass left on it after all the process of harvesting which might result in error in determining the biomass yield of spirulina.

Pomegranate peel extract increased the total chlorophyll content of *A. platensis* (p=0.037), and the highest increase was obtained by 1000 µL PPE. The lowest chlorophyll a was obtained by addition of 10000 µL PPE while the highest was obtained by 1000 µL PPE. Both chlorophyll *a* and *b* take place in photosynthesis, chlorophyll a being the basic molecule and chlorophyll b is the auxiliary molecule. It can be suggested that use of the organic waste PPE in A. platensis cultivation can enhance chlorophyll production. Evaluation of pomegranate peels this way is important for evaluation of waste material and production of chlorophyll rich microalgae.

The lowest carotenoid was obtained by addition of 1000 µL PPE while the highest was obtained by addition of 20000 µL PPE. *A. platensis* is a microalga rich in essential amino acids and fatty acids, vitamin E, carotenoids and minerals (Finamore et al., 2017). Carotenoids are natural pigments found in algae. A. platensis has β-carotene basically but also can have cryptoxanthin and zeaxanthin (Sujatha and Nagarajan. 2013). Human body cannot synthesize carotene and must obtain via foods. The rich carotene sources are basically plants, fruits, vegetables and microalgae (Bakan et al., 2014; Janiszewska-Turak et al., 2016; Rao and Rao, 2007). Even though the carotenoid contents of spirulina in this study were not statistically different from each other, the mathematical increase can be taken into account and enhancement of carotenoid content by PPE addition can be suggested.

4. CONCLUSION

The effects of PPE on *A. platensis* were investigated in this study. The PPE extracts obtained by ethyl acetate did not exhibit antibacterial activity and could dissolve easier, hence the extracts were obtained using ethyl acetate. During the cultivation, at some days, high increase in optical density was observed and this was attributed to the decrease of waster through evaporation. Hence, addition of growth medium to the reactor bottles regularly is suggested for better observation of growth of microalgae. The highest microalgae biomass was obtained by addition of 20000 µL of PPE and it can be suggested that PPE can enhance the biomass production by spirulina. Although carotenoid contents of different groups did not show a statistically significant difference, phycocyanin amounts decreased by increasing PPE concentration. The highest chlorophyll *a* content was obtained by the lowest amount of PPE added and the highest concentration significantly decreased the chlorophyll *a* content. Moreover, 10000 and 20000 µL decreased total chlorophyll contents. PPE addition to *A. platensis* cultures resulted in later harvest time, which can be an economic disadvantage. Therefore, PPE of 1000 µL can be suggested for the evaluation of this valuable organic waste both to produce higher biomass and more bioactive compounds which add value to the microalgae biomass. This study shows that pomegranate peel extracts can be used for spirulina cultivation in different concentrations. This may help the decrease of organic waste dump into the environment and obtaining a high value-added product.

5. ACKNOWLEDGEMENT

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CHAPTER 11

ANALYSIS OF THE TURKISH KIWI SECTOR

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1. INTRODUCTION

Kiwi (*Actinidia spp*.) is a strategic fruit species whose economic value is steadily increasing globally, both for fresh consumption and industrial use. Its high nutritional value and alignment with modern health trends are solidifying its place in global agricultural trade. Turkey has made significant progress in kiwi cultivation, which began in the late 1980s, with total production exceeding 100 thousand tons, making it one of the world's leading producers. This rapid ascent is remarkable as it demonstrates the country's diversification in agricultural production patterns and its considerable export potential.

However, behind this quantitative growth lie critical structural dynamics, such as significant shifts in the dynamics of the global kiwi market over the past two decades and the challenges Turkey's current production profile faces in adapting to these changes. Notably, there has been a distinct demand shift from traditional green kiwi towards new varieties with different taste and appearance profiles. In this context, a country's varietal profile in production stands out as a strategic factor directly influencing its competitiveness in international markets.

For many years, the Black Sea Region was known for its monoculture production pattern due to the dominant role of hazelnuts and tea in its agricultural economy. Factors such as global market fluctuations, the impacts of climate change (especially frost events), and increasing pest pressure have made the regional economy vulnerable due to its excessive reliance on these products. Particularly, the difficulties experienced in tea and hazelnut exports in the 1980s led to discussions about limiting cultivation areas, initiating a search for alternative crops for the region. In this context, the process began when the then Minister of Finance, Adnan Kahveci, requested research into the feasibility of cultivating kiwi in Turkey after being offered the fruit during his visit to Italy. Adaptation studies commenced in the mid-1980s with kiwi seedlings brought from Italy. Kiwi (Actinidia deliciosa), a vine-like woody plant native to the forests of East and South China, named after the bird that feeds on it, has seen significant increases in production in recent years, despite intense demand for its consumption (Samancı, 1990).

Upon confirmation that kiwi adapted well to the region, this fruit was seen as a promising alternative and its widespread cultivation was supported. Although initial challenges included a lack of producer knowledge and the absence of a marketing chain, local producer associations were established under the leadership of technical organizations and chambers of agriculture. Cold storage facilities were planned, and a marketing chain was created. Thus, kiwi, which was initially marketed locally, expanded to national markets as production volumes increased, and Turkey became an important production center for a new fruit species.

The aim of this study is to analyze the Turkish kiwi sector from a multi-layered perspective. In this framework, the study will: (i) present Turkey's national production and foreign trade performance using data from the last ten years, (ii) critically evaluate Turkey's current production profile in light of changing consumer demands and varietal trends in the global market, and (iii) deeply examine the reflections of these general dynamics in the Black Sea Region, one of the country's most significant kiwi production basins, through a case analysis and SWOT analysis. This holistic approach aims to provide a comprehensive foundation for understanding the current state of the sector and making strategic inferences for the future. The report aims to go beyond quantitative achievements, focusing on qualitative and strategic adaptation issues that will determine the sector's future position, revealing potential fragilities and the necessity for transformation behind a success story.

Figure 1: Ripe kiwifruit grown on a trellising system in an orchard

2. GENERAL PANORAMA OF THE TURKISH KIWI SECTOR: PRODUCTION AND TRADE DYNAMICS

Kiwi (*Actinidia deliciosa*) cultivation in Turkey began in 1988 with the first seedlings brought into the country by the Yalova Atatürk Horticultural Research Institute, and adaptation studies were successfully completed (Ateş et al., 2007). In the following years, kiwi production rapidly spread, especially in certain regions, becoming an important agricultural branch for both domestic consumption and foreign trade.

Figure 2: Major kiwi varieties demanded in the global market (green, yellow, and red-fleshed)

2.1. Production Analysis: National and Regional Distribution

Kiwi production in Turkey generally showed a significant growth trend between 2014 and 2023. Taking 2014 as the base year, the "Production Area (ha)" increased by approximately 93.56% by 2023, indicating that the land allocated for kiwi cultivation nearly doubled. This expansion can be linked to factors such as increased demand or profitability for kiwi. In parallel, the "Production Quantity (tons)" also showed a remarkable increase; it peaked at approximately 216.97% in 2022 but slightly decreased to 182.59% in 2023. This suggests that the increase in production area in previous years directly reflected in the production quantity, but there might have been a fluctuation in yield in the last year due to climatic conditions or other factors.

When "Yield (kg/decare)" values are examined, a general upward trend is observed compared to 2014, although the rate of increase has been more moderate than the increase in production area and quantity. The highest yield increase was recorded in 2022 with 66.71%, but this rate dropped to 46.00% in 2023. This indicates that despite significant increases in production area and total output, productivity per decare increased relatively slower or experienced declines in some years. This can be explained by factors such as the spread of more efficient production techniques, the adaptation of new and more productive kiwi varieties, or the wider application of good agricultural practices.

Figure 3: A View of Increasing Area and Productivity in Turkish Kiwi Production[4]

Table 1: Kiwi Production Area and Quantity by Years in Turkey (2014-2023)

Year	Production Area (ha)	Production Area (ha) % Change	Production Quantity (ton)	Production Quantity (ton) % Change	Yield (kg/decare)	Yield (kg/decare) % Change
2014	2219	0.00	31795	0.00	1433	0.00
2015	2411	8.65	41640	31.00	1727	20.52
2016	2487	12.08	43950	38.22	1767	23.31
2017	2744	23.66	56164	76.65	2047	42.85
2018	2990	34.74	61920	94.75	2071	44.52
2019	3067	38.21	63798	100.67	2080	45.15
2020	3261	46.96	73745	132.06	2261	57.78
2021	3884	74.94	86362	171.60	2224	55.20
2022	4219	90.13	100772	216.97	2389	66.71
2023	4295	93.56	89831	182.59	2092	46.00

Source: TÜİK, Crop Production Statistics (2024)

However, the decrease in production quantity and yield in 2023 indicates that additional research and practices may be required for sustainable production and increased productivity. Overall, the kiwi sector in Turkey has a

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4 https://www.tv100.com/turkiyenin-en-fazla-kivi-uretim-alanina-sahip-ilcesi-carsambada-kivi-hasadi-basladi-haber-708697

dynamic structure that continues to grow in terms of production area and quantity.

Kiwi (*Actinidia deliciosa*) cultivation in Turkey began in 1988 with the first seedlings brought from Italy by the Yalova Atatürk Horticultural Research Institute, and the adaptation studies were successfully completed. In the following years, kiwi production rapidly spread, especially in the Black Sea and Marmara regions, becoming an important agricultural branch for both domestic consumption and foreign trade (Harman, 2014; Cangi and İslam, 2003).

Marmara Region: The Marmara Region is the leader in kiwi production in Turkey. Yalova is considered the kiwi capital of the country, and as of 2022, the year Turkey peaked at 100,772 tons, Yalova accounted for approximately 27% of the total production. However, as of 2024, this rate has decreased to 15.6%. Factors such as suitable climate and soil structure, the presence of research institutes in the region, and the experience of local farmers play a significant role in Yalova's leadership. In the Marmara Region, Bursa follows Yalova and ranks second overall in Turkey, taking the first place with 20.2% according to 2024 data (TÜİK 2024).

Black Sea Region: Kiwi production in the leading provinces of the Black Sea Region has shown significant changes in both cultivation area and production quantity between 2014 and 2024 (Table 2). While kiwi cultivation areas and, consequently, production quantities have shown a considerable decreasing trend in some provinces like Artvin and Giresun, provinces such as Sakarya and Samsun have vastly increased their cultivation areas and production quantities, becoming new centers of kiwi production in the region (TÜİK, 2024). For example, Sakarya's production area has increased more than 10 times, and its production quantity has increased by approximately 58 times (TÜİK, 2024). Similarly, Samsun has also recorded substantial growth in both cultivation area and production quantity (TÜİK, 2024). While Ordu has steadily increased its cultivation area and production, the fact that provinces like Rize and Trabzon have increased their production quantities despite a partial decrease in cultivation areas suggests an increase in yield per unit area and the adoption of more modern agricultural techniques in existing areas (TÜİK, 2024). This situation indicates that kiwi farming in the Black Sea Region is undergoing a dynamic transformation in terms of geographical distribution and production capacity, with some provinces specializing in kiwi production

(TÜİK, 2024). While Bursa ranks first in overall kiwi production in Turkey, Yalova has dropped to second place. Samsun has moved up to third place (2024 TÜİK). It is stated that kiwi is seen as an alternative crop to hazelnuts in the Black Sea Region, and kiwi production is increasing (Harman, 2014). However, particularly in the Eastern Black Sea (Artvin, Rize, Trabzon, and Giresun) provinces, kiwi has lost its potential as an alternative or additional income-generating crop to tea and hazelnuts. It is understood from the increasing shares of provinces like Samsun and Sakarya in production that kiwi, like hazelnuts, is rapidly spreading towards the Central and Western Black Sea regions where topographical and ecological conditions are more favorable. This situation suggests that kiwi is not so much an alternative or additional income-generating crop for the Eastern Black Sea Region, but rather a new fruit species introduced into our country's production. Kiwi has found its place more in flatland areas suitable for vegetable, grain, and legume production, rather than in hazelnut and tea production areas.

Aegean and Mediterranean Regions: While kiwi production in Turkey is concentrated in the Marmara and Black Sea regions, production in the Aegean and Mediterranean regions is at a more limited level (Sincik et al., 2018; Evecen et al., 2021). The Aegean Region, in particular, accounts for only approximately 1% of total kiwi production (Sincik et al., 2018). In the Mediterranean Region, kiwi cultivation is quite restricted, mostly seen in small-scale gardens along the coastline and in experimental productions (Evecen et al., 2021). However, the high plateaus in the foothills of the Taurus Mountains in Mersin hold significant potential for kiwi production. Considering kiwi's requirement for low temperatures, especially the high-altitude plateaus and transit zones of the Mediterranean Region provide suitable climatic conditions for kiwi cultivation. Indeed, Mersin ranks fourth in Turkey's kiwi production with 11,933 tons (TÜİK 2024). This situation indicates the future development potential of kiwi farming in these regions.

Table 2: Kiwi Cultivation Area (decares) and Production Quantity (Tons) in Selected Provinces of the Black Sea Region by Year

Year	Kiwi Cultivation Area (decares)						
	Artvin	Giresun	Ordu	Rize	Sakarya	Samsun	Trabzon
2014	879	2123	2936	3632	235	1572	1653
2018	359	2050	2978	3520	1985	2778	1454
2021	310	652	3709	3338	2568	3757	1330
2024	204	680	3914	3279	**2668**	5646	1039
Year	**Production Quantity (Tons)**						
2014	562	621	1825	4584	132	876	1009
2018	498	2024	7336	5286	3440	5041	1955
2021	435	1703	8530	5621	5292	9611	1902
2024	297	1611	8560	5729	7679	13599	1517

Source: TÜİK (2024) data compiled.

2.2. Trade Analysis: Exports, Imports, and Foreign Trade Balance

Parallel to the significant increase in kiwi production, Turkey has also become a prominent actor in foreign trade (Er, 2023; TOBB, 2023; Tarım Kredi Kooperatifleri, 2023). Turkey, which is both an exporter and an importer, has a foreign trade balance that fluctuates annually. The country's kiwi exports have shown a considerable increase in both quantity and value over the last decade. Notably, kiwi exports, which were at levels of 5-6 million dollars in 2015, reached 35 million dollars as of 2023, marking a growth of over 600%. This increase has been influenced by the rise in production volume and the success of Turkish exporters in entering international markets. The quality, shelf life, and taste of Turkish kiwi are important factors increasing demand in international markets. Major export markets include the Russian Federation (the largest buyer), European Union countries such as Spain, the Netherlands, and Germany, and in recent years, India and Middle Eastern countries (TOBB, 2023).

Despite being a significant kiwi producer, Turkey imports kiwi during periods when domestic demand cannot be fully met by local production or when local produce is not yet available on the market (Sincik et al., 2018). The primary import sources are Iran, due to its low production costs and geographical proximity, and Chile, a Southern Hemisphere country that produces during the off-season (Sincik et al., 2018). Discussions regarding the quality and storage standards of Iranian kiwi occasionally occur in the market (Kendirli & Çetin, 2018). Despite the increase in domestic production, imports continue due to vibrant domestic consumption, and especially during winter months, imports from Iran pose significant competition for local producers (Sincik et al., 2018).

Overall, in the last decade, Turkey has transitioned from being a net importer to a net exporter in kiwi trade. Particularly after 2020, the strong increase in production allowed exports to surpass imports (Zenginbal, 2012; TOBB, 2023). However, low-priced imports from Iran can sometimes negatively impact the trade balance (Sincik et al., 2018). This situation indicates that despite the quantitative improvement in Turkey's foreign trade balance for kiwi, it is still influenced by price-oriented competition, and local producers face import pressure during certain periods. In this context, the sector needs to not only increase its production volume but also improve its cost-effectiveness and its ability to maintain product quality throughout the year.

3. GLOBAL KIWI MARKET AND TURKEY'S VARIETY PROFILE

The dynamics of the global kiwi market have undergone significant transformation over the last two decades. A distinct shift in consumer preference is observed from traditional green kiwi (Actinidia deliciosa) varieties towards new varieties with different organoleptic properties, such as yellow-fleshed (Actinidia chinensis) and miniature (baby kiwi) types (Wang et al., 2021). The main driving forces behind this change include increasing health consciousness, demographic factors, and the marketing strategies of global retail chains (Gil et al., 2022; Harker et al., 2006).

3.1. Major Kiwi Varieties in Global Trade Demands

The global kiwi market has experienced a significant transformation after 2010, driven by varietal breeding efforts and shifts in consumer trends (Ferguson & Huang, 2007). In this transformation, the commercialization of new species, alongside traditional green kiwis, has been decisive. The main kiwi groups shaping the market are:

Yellow-Fleshed (Gold) Kiwi Varieties (Actinidia chinensis): This group comprises varieties with less hairy or completely smooth skin, a sweeter taste profile, and lower acid content. The fruit flesh color ranges from golden yellow tones and contains aromatic compounds reminiscent of tropical fruits (Montefiori et al., 2005).

- ✓ **'Zespri SunGold'** (Variety code: ZSY002): This proprietary variety, originating from New Zealand, holds a dominant position in the global market due to its licensed production model. It stands out for its fruit quality (high Vitamin C and carotenoid content) and brand strategy (Zespri Annual Report, 2023).
- ✓ **'Jintao'** (Jingold™ brand name): This variety, bred in Italy, has Protected Designation of Origin (PDO) protection in Europe. It demonstrates significant superiority in **sensory analyses** compared to traditional varieties (Huang, 2016).

Red-Fleshed Kiwi Varieties: These varieties exhibit red-purple coloration in their fruit flesh due to anthocyanin accumulation. The Chinese variety 'Hongyang' is considered a functional food because of its antioxidant capacity (Wang et al., 2020).

Hard-Cored Mini Kiwis (Actinidia arguta): Also known as "kiwi berry," this species is the size of a large grape (5-15 g), with thin, hairless skin. It's preferred in the snack industry due to its ability to be consumed with the skin and its high nutritional value (Latocha et al., 2021).

3.2. Major Kiwi Varieties Cultivated in Turkey: 'Hayward' Dependency

Kiwi cultivation in Turkey largely shows a monoculture structure based on a single variety. More than 95% of commercial kiwi orchards consist of the 'Hayward' variety, belonging to the species Actinidia deliciosa (Testolin & Ferguson, 2009; Ferguson & Huang, 2007). This situation creates a vulnerable structure for Turkey's kiwi production due to a lack of diversity and potential risks (climate change, disease pressure, market fluctuations).

Prominent Features of the 'Hayward' Variety

1. Fruit Quality and Market Value

- **Fruit characteristics:** 'Hayward' is large, oval-shaped, with bright green flesh and an aromatic taste. Fruit weight typically ranges between 80-120 g, a size preferred by consumers (Ferguson, 2016).

- **Market demand:** Globally, it's recognized as the standard green kiwi variety and holds a dominant position in exports (Huang, 2014).

2. Storage and Shelf Life

- It can last for up to 6 months in cold storage (0°C, 90-95% humidity) (Shahkoomahally and Ramezanian, 2015).
- Its long shelf life is commercially advantageous, offering flexibility in logistics and export (Özkan and Yılmaz, 2017).

3. Productivity and Adaptation

- Due to its high yield potential, it's successfully cultivated in Turkey's Black Sea, Marmara, and Aegean regions (Korkmaz and Özzambak, 2020).
- Its partial tolerance to climate change supports sustainable production (Akbulut and Sümer, 2018).

4. Pollinator Varieties

- Since 'Hayward' is a female variety, it needs male varieties for pollination. The most commonly used pollinators are:
- 'Matua' (compatible with early flowering)
- 'Tomuri' (late flowering, synchronized with 'Hayward') (Ferguson and Bollard, 1990; Özkan and Yılmaz, 2017)

Alternative Variety Trials and Limited Success in Turkey

- ✓ Other green kiwi varieties like 'Bruno', 'Monty', and 'Abbott' have been tested in Turkey, but they lagged behind 'Hayward' in terms of yield, fruit quality, and storage durability (Korkmaz et al., 2023).
- ✓ Yellow-fleshed (Actinidia chinensis) varieties (e.g., 'Hort16A' / Zespri® Gold) have been tested in limited areas in Turkey but have not become widespread due to climate adaptation challenges and disease susceptibility. [[5]]

[5] https://kocaelibitkileri.com/actinidia-chinensis-var-deliciosa/

Risks of 'Hayward' Dependency and Sectoral Impacts

1. **Lack of Intellectual Property and Low Margin Problems**

- Since 'Hayward' is a public domain variety, it limits Turkey's opportunity to develop value-added products in the global market.
- While competitors like New Zealand (Zespri®) and Italy target high-profit foreign markets with licensed proprietary varieties, Turkey remains confined to price-driven competition (Zespri International Limited., 2023).

2. **Climate Change and Biotic Stress Threats**

- Monoculture farming can create vulnerability to pathogens like Pseudomonas syringae (bacterial canker) (Rossetti and Balestra 2008).
- Rising temperatures can affect 'Hayward's chilling requirement, leading to decreased yields (Bostan and Günay, 2014).

3. **Lack of Market Diversification**

- Research by Jaeger and Harker on consumer preferences has examined how factors such as kiwi color, taste, and ease of consumption influence consumer behavior. Studies show that new kiwi varieties like red-fleshed 'Hongyang' are preferred by consumers at higher prices. Turkey's production structure struggles to respond quickly to these demands (Harker et al., 2006).

While Turkey's 'Hayward' dependency ensures stable production in the short term, in the medium to long term, it is necessary to develop diversification strategies (new patented varieties, organic production, yellow/red kiwi varieties). In this context:

- ✓ Local breeding programs (TAGEM, universities) should be supported.
- ✓ Branding in export markets (geographical indications, premium segment) should be targeted.
- ✓ Climate-resilient varieties (those with low chilling requirements) should be tested.

4. Black Sea Region Kiwi Sector: An In-depth Case Analysis

The Black Sea Region stands out as one of Turkey's significant kiwi production centers. The region draws attention with its concentrated kiwi production, especially in the provinces of Samsun, Ordu, and Rize. Kiwi has been adopted as an alternative to hazelnut and tea farming in the region and has become an economically important crop. A portion of Turkey's total kiwi production is sourced from this region (Table 2). Çarşamba district in Samsun has the country's largest kiwi production area, accounting for 12.88% of Turkey's total kiwi production [[6]] (Zenginbal, 2012).

4.1. Regional Production Dynamics and Producer Profile

The subtropical climate characteristics of the Black Sea Region, with its mild winters and rainy summers, naturally meet the kiwi's water and humidity requirements, providing a significant advantage for cultivation. This ecological suitability has led to a steady increase in production areas over the last decade. The conversion of some hazelnut and tea gardens to kiwi plantations has played an important role in this increase (Akbulut et al., 2015).

Kiwi producers in the Black Sea Region are generally small-scale family businesses. The main factors shaping producer tendencies and profiles are:

- ✓ Economic Motivation: Kiwi cultivation in the Black Sea Region has emerged as an economically attractive alternative for producers in recent years. The primary driving force behind this trend is the significantly higher net income per decare provided by kiwi production compared to hazelnut production. Economic comparisons show that kiwi can provide approximately 3 to 5 times more income than hazelnuts (Yavuz et al., 2015). This economic superiority has influenced the transformation of the region's agricultural production pattern; notably, there has been a significant increase in cultivation areas with the conversion of some hazelnut orchards into kiwi plantations. Indeed, the mild winter conditions and rainy summer climate of the Black Sea Region provide a natural advantage in meeting kiwi's high humidity and water needs, supporting this transformation (Akbulut et al., 2015). However, there are some

[6] https://www.yildizhaber.com.tr/karadenizde-kivi-hasadi-131675-haberi#google_vignette

structural constraints preventing this economic motivation from fully translating into producer incomes. In particular, deficiencies in marketing infrastructure, non-standard production, and lack of technical knowledge prevent the full realization of this potential. Therefore, for the sustainable growth of the kiwi sector in the region, both the development of production techniques and the strengthening of organized marketing channels are necessary.

- ✓ Farm Structure: Kiwi farms in the Black Sea Region generally consist of small and fragmented plots ranging between 2 and 10 decares, consistent with the region's traditional agricultural structure (Doğan et al., 2025). This structure limits the use of modern agricultural machinery, necessitating human labor for all cultural practices, which increases labor costs and limits production efficiency (Ordu Ticaret Borsası, 2020; Turna et al., 2003). Therefore, to address structural inefficiencies in the sector, policies such as land consolidation and mechanization support need to be prioritized. Thus, structural reforms such as land consolidation, cooperativization, and mechanization support are required for sustainable growth in the sector.
- ✓ Age and Experience: In addition to young farmers new to kiwi production, there is also an older group of producers with experience in hazelnut production but new to kiwi. Intergenerational differences are observed in access to technical knowledge and adoption of new technologies.
- ✓ Risk Perception: One of the main risk factors shaping decisions regarding kiwi cultivation in the Black Sea Region is the high initial investment costs. Infrastructure requirements, particularly the installation of trellising systems, create a significant financial burden for small-scale producers. Furthermore, late spring frosts coinciding with the plant's flowering period are considered a climatic risk factor that directly threatens yields. These risks have a significant deterrent effect on producers' investment decisions (Pekdemir and Kılıç, 2020).

4.2. SWOT Analysis: Strengths, Weaknesses, Opportunities, and Threats

A detailed SWOT analysis (Strengths, Weaknesses, Opportunities, Threats) has been conducted to strategically assess the current state and future of the kiwi sector in the Black Sea Region. This analysis provides a roadmap for policymakers and producers by enabling a comprehensive view of the sector's internal capabilities and external environmental factors.

Table 3: SWOT Analysis of the black sea region kiwi sector

STRENGTHS - Internal Advantages	WEAKNESSES - Internal Disadvantages
1. **Ideal Ecological Suitability:** The region's high humidity, regular rainfall, and acidic soil structure provide an excellent environment for kiwi.	1. **Fragmented and Sloped Land Structure:** Small and scattered parcels that make mechanization impossible and increase labor costs.
2. **High Unit Area Income:** Proven significantly higher profitability compared to hazelnuts, serving as a strong motivation for producers.	2. **Inadequate Storage and Marketing Infrastructure:** Lack of modern cold storage facilities and weak producer organizations.
3. **Product Quality and Flavor:** The regional climate ensures high desirable taste, aroma, and Vitamin C content in kiwi.	3. **High Initial Investment Cost:** Trellising system and seedling costs are a significant barrier for small farmers.
OPPORTUNITIES - External Factors	THREATS - External Factors
4. **Increasing Producer Experience:** Accumulated knowledge and experience in kiwi cultivation over the years.	4. **Dependency on a Single Variety ('Hayward'):** Inability to respond to market diversification and new consumer demands (yellow kiwi, etc.).
1. **Growing Organic Farming Market:** The region's ecology offers great potential for transitioning to organic production, which can be sold at premium prices.	1. **Climate Change and Extreme Weather Events:** Increased risk of late spring frosts, heavy rainfall, and storms.
2. **Branding and Geographical Indication:** Potential to increase added value with "Black Sea Kiwi" brand and geographical indication registration.	2. **Global Competition and Price Pressure:** Price competition, especially from low-cost producers like Iran.
3. **Increasing Domestic Consumption and Healthy Lifestyle Trends:** Rising domestic demand for kiwi due to increasing health consciousness.	3. **Rising Input Costs:** Global and national increases in fertilizer, pesticide, energy, and labor costs threatening profitability.
4. **Rural Tourism (Agro-tourism) Integration:** Opportunity to transform kiwi orchards and harvest into a tourist attraction.	4. **Emergence of New Diseases and Pests:** Risk of globally spreading diseases like kiwi bacterial canker (*Pseudomonas syringae actinidiae* - Psa) reaching the region.

Evaluation of SWOT Analysis: The SWOT table reveals that the Black Sea Region's kiwi sector is complex. On one hand, it possesses very strong internal dynamics like ideal ecological conditions and high profitability. On the other hand, it struggles with chronic weaknesses such as structural inefficiencies due to land fragmentation and inadequate infrastructure (Table 3).

Strategically, the most logical approach is to leverage strengths to capitalize on opportunities. For instance, the region's ecological suitability and high product quality can be a powerful tool for pursuing organic farming and branding opportunities. An organically certified and geographically indicated "Black Sea Kiwi" would significantly shield producers from the threat of global price competition. This highlights the potential for creating added value by combining the region's natural advantages with market demands.

However, weaknesses are the biggest obstacle to realizing these opportunities. To capitalize on branding, a consistent supply of standard quality products in significant volume is needed, yet fragmented land structure and lack of organization make this difficult. Similarly, insufficient storage facilities undermine producers' bargaining power, leaving them more vulnerable to rising input costs. This situation clearly shows that structural weaknesses pose a serious barrier to the region's full potential.

The most concerning scenario involves the convergence of weaknesses and threats. For example, dependency on a single variety poses a major competitive threat when new trends like yellow kiwi emerge in the global market. Simultaneously, the small farm structure prevents producers from investing in expensive protection systems against climate change threats like frost. These intersections highlight the urgent need for comprehensive and holistic interventions to ensure the sector's sustainability.

5. STRATEGIC JUNCTIONS AND FUTURE SCENARIOS

The analyses in the preceding sections clearly show that while the Turkish kiwi sector has achieved quantitative success, it stands at a qualitative and strategic crossroads. This section will delve into the underlying causes of critical issues previously only mentioned in passing and discuss possible future scenarios for the sector.

Addressing the "variety problem" in the kiwi sector merely as a simple dilemma between 'Hayward' and 'yellow kiwi' means overlooking the depth of the issue. The real matter at hand is intellectual property (IP) management and branding strategies that are fundamentally changing the dynamics of global fruit trade. These strategies form the basis for gaining competitive advantage and creating added value in the world market.

The Anatomy of the Zespri Model: An Integrated Success Story: The Zespri Model stands out as a success story in the global kiwi market. This model not only focuses on developing high-quality kiwi varieties but also includes comprehensive plant breeding programs sustained through public-private sector collaboration. At the core of the model lies the dissemination of varieties protected by intellectual property rights (e.g., 'ZESY003' and 'ZESH004') through a global licensing system. This system is supported by strict control mechanisms that ensure the same quality standard at every production point. Zespri integrally manages the entire process of its branded kiwis, from production to marketing, making it possible to offer uniformly high-quality products to consumers worldwide. Furthermore, high-budget global marketing strategies that have made the brand synonymous with "kiwi" have transformed Zespri into an exemplary model for turning agricultural products into brand value (Zespri Group Limited, 2021; USPTO, PP22292 & PP22276).

Turkey's Current Situation: Price-Driven Competition and Exclusion: Turkey's kiwi sector, conversely, is in a position diametrically opposite to the Zespri Model. The 'Hayward' variety, predominantly produced in Turkey, has been in the public domain for over 100 years and carries no intellectual property rights. Production is mostly carried out by thousands of small and unorganized farmers without any common quality standards. Exports are generally made under generic labels like "Turkish Kiwi" and with a solely price-driven competitive approach. This situation clearly demonstrates that Turkey's dependency on 'Hayward' is more than a simple preference for taste or color; it's an exclusion from the intellectual property dynamics that control the most profitable segments of the global market. This exclusion pushes Turkey into a "price-taker" position in the global market, severely limiting its strategic autonomy.

Turkey's Strategic Choice: At this critical juncture, Turkey faces two strategic options: either create its own unique model or become part of the global system.

- ✓ Creating its own model requires establishing a national "Kiwi Research and Development Center of Excellence," financing decades-long breeding programs, and finding the patience and resources needed to introduce a "Turkish Kiwi Brand" to the global market. This path offers full strategic autonomy and high value-added potential in the long run.

- ✓ Becoming part of the global system involves entering into licensing agreements with Zespri or other global players to produce their patented varieties in Turkey and market them under their brands. While the second option might seem like a quicker solution, it risks making the sector entirely dependent on external entities. Turkey's choice here will directly determine its future position and economic gains in the kiwi sector.

The structural weaknesses noted in the SWOT analysis, such as *"fragmented land structure"* and *"lack of organization,"* stem not only from economic choices but also from deep-rooted sociological and legal factors. This presents a significant barrier limiting the potential of Turkey's agricultural sector.

The Impact of Inheritance Law on Agricultural Lands: Turkey's inheritance law, based on the Civil Code, leads to significant reduction in the size of agricultural lands as they are divided among heirs with each generation. For example, a 50-decare orchard inherited from a grandfather can, after a few generations, turn into economically unviable 2-3 decare plots. This situation is the most fundamental structural impediment hindering mechanization, productivity, and modern agricultural practices. It's clear that this problem will persist unless legal regulations preventing land fragmentation (e.g., transfer to a "competent heir" or land consolidation) are effectively implemented. This legal and social structure prevents agricultural enterprises from benefiting from economies of scale and thwarts modernization efforts.

"Old" vs. "New" Cooperative Models: A Paradigm Shift: Unfortunately, the idea of *cooperatives in Turkey* has, in the past, led to distrust and a reserved stance among producers due to political interventions, inefficient management, and irregularities. However, for the sector to overcome its current challenges, it must adopt a **"***new generation cooperative***"** approach, moving beyond "old-style" cooperative models.

New generation cooperatives, unlike traditional models:

- ✓ Are managed by professional administrators.
- ✓ Are not limited to merely collecting and selling products but also focus on value-added activities such as branding, marketing, R&D, and technology adaptation.
- ✓ Offer comprehensive services to their members, including technical consultancy, access to finance, and risk management.
- ✓ Operate with a transparent, accountable, and entirely market-oriented approach.

It is crucial for the state to support the establishment of these new generation, professional, and market-oriented cooperative models with **special incentives**. This support will not only promote an economic organizational model but also highlight the necessity of a cultural and institutional transformation to overcome past negative experiences.

5.3. Defining a National Strategy: Commodity Producer or Value-Added Player?

In light of all analyses, two main future scenarios can be drawn for the Turkish kiwi sector. It is critically important for the government and sector stakeholders to consciously choose one of these scenarios for the sector's future success. Defining a strategic direction will enable the sector to escape its current uncertainty and embark on a sustainable growth path.

Scenario A: Becoming an "Efficient Commodity Producer": In this scenario, Turkey aims to become one of the world's most efficient and low-cost producers of 'Hayward' and similar public domain kiwi varieties. The core strategy is based on cost minimization. This approach requires accelerating land consolidation projects, strengthening logistics and storage infrastructure with state support, and investing in mechanization and automation technologies to increase efficiency. Furthermore, to compete effectively on price with rivals like Iran, meticulous optimization of all production processes is essential for this scenario.

- ✓ **Outcome:** Turkey would be positioned as a "price taker" in the global market. While profitability might be low in this case, production volume could be high. The biggest risk is extreme vulnerability to global price fluctuations and exposure to external shocks. Even if this scenario cannot be fully realized due to existing structural problems, it could lead the sector to remain in a low-margin cycle.

Scenario B: Becoming a "Value-Added Niche Player": In this scenario, Turkey aims to compete with countries like Italy and New Zealand in terms of quality and brand. The strategy is based on value maximization. This path necessitates serious and long-term investment in a national variety breeding program and making organic and good agricultural practices the sector standard. Moreover, creating a strong brand identity and an effective marketing strategy for "Turkish Kiwi" or sub-brands (e.g., "Black Sea Organic Kiwi"), and focusing on high value-added, niche markets, are the cornerstones of this scenario.

- ✓ **Outcome:** Turkey gains the potential to become a "price maker" in the global market. Profitability could be high, but market share might be limited to a more niche segment. Risks in this scenario include the requirement for high initial investment and the long, challenging nature of the branding process.

Current State of Strategic Uncertainty: Currently, the Turkish kiwi sector is stuck between these two scenarios, in a position of strategic uncertainty. Due to structural problems, the sector can neither fully become an efficient commodity producer nor a value-added player due to a lack of R&D and branding. This strategic uncertainty is a fundamental problem that prevents the sector from fully realizing its potential and threatens its sustainability. Without a clear strategic direction, it seems difficult for the sector to maintain its current successes and strengthen its position in the global market.

6. CONCLUSION AND STRATEGIC RECOMMENDATIONS

While the Turkish kiwi sector has achieved quantitative growth, it stands at a critical juncture in terms of qualitative transformation and strategic alignment. All analyses presented in this study suggest that Turkey's potential leans more towards becoming a "Value-Added Niche Player," corresponding to Scenario B. The ecological advantages offered by the Black Sea Region, in particular, provide a unique foundation for value-creation strategies like organic farming and geographical indications. The current strategic uncertainty is unsustainable; although choosing this path requires a more challenging and long-term vision, it is the only realistic strategy to lead the Turkish kiwi sector to a respected, profitable, and sustainable future in the global market.

In line with this, the following multi-layered, action-oriented strategic recommendations are presented to shape the sector's future:

Diversification and R&D: Turkey must break its 'Hayward' dependency. Research institutes under the Ministry of Agriculture and Forestry, along with universities, should accelerate projects aimed at determining the adaptation performance of public domain yellow kiwi varieties demanded in the global market, such as 'Jintao' and 'Soreli', and potential new candidates across Turkey's different ecological regions. Furthermore, opportunities for international cooperation and licensing agreements should be explored to enable the production of patented and licensed varieties like 'Zespri SunGold' in Turkey. Long-term investments should be made in developing unique yellow or red-fleshed kiwi varieties that meet market expectations through domestic breeding programs, utilizing Turkey's rich genetic resources. These steps, by embracing the importance of intellectual property, *will enable the sector to access high value-added markets*.

Organization and Infrastructure: As seen in the Black Sea example, the disadvantages of fragmented and small-scale producer structures can only be overcome through strong producer associations. Producer associations should play a key role in joint marketing, input supply, machinery pool usage, and most importantly, establishing a modern cold chain to prevent post-harvest losses. Public policies should support these organizations and infrastructure investments (especially low-interest loans and grant programs for licensed warehousing and cold chain infrastructure). Promoting new generation, professional producer association models is vital for overcoming the sector's structural problems.

Branding and Market Development: Turkey must transition from an image of "cheap 'Hayward' supplier" to that of a "producer of quality and diverse kiwi." A high value-added brand identity should be created in both domestic and export markets, utilizing tools like organic production potential and geographical indications. Support for certification and consultancy should be provided for transitioning to organic farming, and Geographical Indication registration processes should be accelerated. These strategies will enable the product to compete not just on volume, but also on quality, sustainability, and uniqueness.

Risk Management and Technical Knowledge Dissemination: The scope of insurance policies against frost risk under TARSİM (Agricultural Insurance Pool) should be expanded, and premium subsidies increased to make them more attractive to producers. Additionally, SME-style supports should be provided for frost control methods. Educational and extension activities are needed for producers on how diversification can reduce market risks and increase profitability. Provincial/District Directorates of Agriculture and Forestry and universities should intensify on-farm practical training programs on modern pruning techniques, balanced fertilization, frost control methods, and organic farming practices. These steps will make producers more resilient to climatic and economic risks and increase overall productivity.

A coherent and holistic approach to these interconnected recommendations constitutes the only realistic roadmap to position the Turkish kiwi sector among the leading global players, not just in quantity, but also in value. This transformation requires the shared vision and effort of all stakeholders, from producers to policymakers, researchers to exporters.

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CHAPTER 12

SILVER NANOPARTICLES AND THEIR CURRENT BIOMEDICAL APPLICATIONS

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1. INTRODUCTION

Nanotechnology is the study of materials at the nanoscale, namely within the dimensions of 1 to 100 nanometers. The realm of nanoscience diverges from the larger world we encounter in our daily existence, focusing on various facets of nanotechnology. As the length scale of materials diminishes, surface-area effects gain significance and quantum effects arise, resulting in alterations to the characteristics of materials. The number of atoms relevant to an object's surface rises due to the dense structure of metals . Nanoparticles (NPs) exhibit remarkable variability in their chemical composition, dimensions, charge, morphology, and surface area(Abbas et al., 2024).

Silver nanoparticles (AgNPs) are progressively utilized throughout diverse domains, including medicine, food, healthcare, consumer products, and industrial applications, owing to their distinctive physical and chemical characteristics. These encompass optical, electrical, thermal, high electrical conductivity, and biological characteristics . Owing to their unique properties, they have been utilized in various applications, including as antimicrobial substances in industrial, household, and healthcare products, consumer goods, medical instrument coatings, optical sensors, cosmetics, the pharmaceutical sector, the food industry, orthopedics, drug delivery, and diagnostics and as anticancer agents, ultimately augmenting the tumor-eradicating effects of anticancer drugs(Zhang et al., 2016).

Silver nanoparticles are particularly intriguing for biomedical research. Advancements are occurring in this interdisciplinary domain involving the utilization of nanoparticles. The contemporary biological potential of AgNPs includes their implementation in therapeutically advanced individualized healthcare services. Silver nanoparticles (AgNPs) exhibit significant properties and remarkable potential for the advancement of novel antimicrobial agents, drug delivery systems, detection and diagnostic platforms, coatings for biomaterials and medical devices, materials for tissue repair and regeneration, strategies for complex health conditions, and improved therapeutic options. Considering the remarkable medicinal potential of AgNPs, considerable efforts have been devoted to elucidating the intricate mechanisms of their biological interactions and their toxicological effects(Burdușel et al., 2018).

1.1 Preparation Silver Nanoparticle

A variety of methods are utilized for the production of silver nanoparticles, encompassing physical, chemical, and biological synthesis. It is important to acknowledge that each strategy possesses distinct advantages and downsides. In the biological manufacture of silver nanoparticles, the organism functions as a capping agent, reducing agent, or stabilizing agent, converting Ag^+ to Ag^0. Biological approaches utilizing natural products derived from microorganisms and plant sources have gained favor in recent years due to their cost-effectiveness, high yields, and minimal toxicity to humans and the environment . The subsequent sections delineate various approaches for the manufacture of silver nanoparticles(Almatroudi, 2020a).

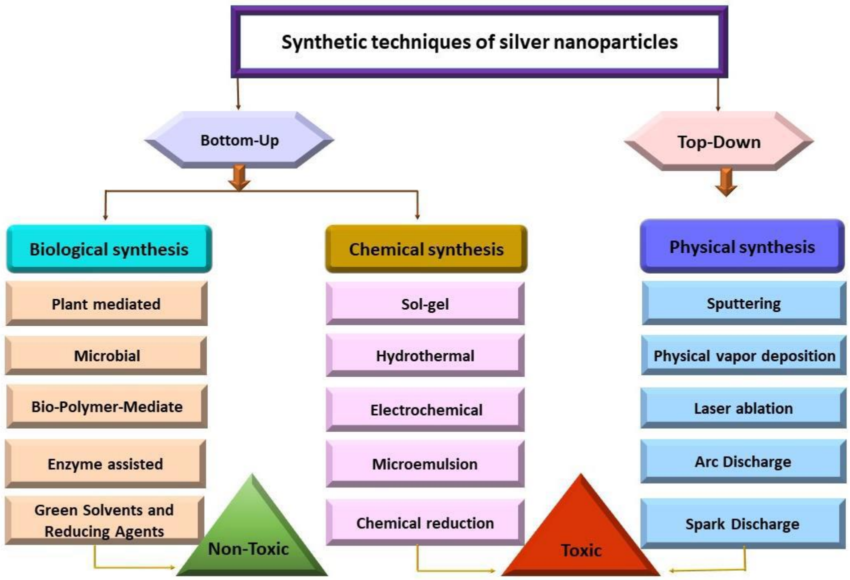

Figure 1: General scheme of Preparition of Silver Nanoparticle (Abbas et al., 2024)

Figure 1 summarizes the various approaches and methods used for silver nanoparticle synthesis. As technology and materials science advance, the number of methods listed in the table increases. This is to achieve high-yield, stable, multifunctional, and environmentally friendly production procedures using fewer materials. Top-down and bottom-up methodologies are essential strategies for nanoparticle (NP) synthesis. Top-down approaches transform bulk materials into nanoparticles without chemical additions, yielding nanoparticles with dimensions between 10 and 100 nm. These nanoparticles frequently demonstrate elevated purity and uniform diameters. Nonetheless, they are devoid of stabilizers or capping agents, which are essential for averting agglomeration. Bottom-up techniques synthesize nanoparticles (NPs) by assembling atoms and molecules from smaller constituents, including self-assembly of monomer molecules, enzym asisted, redction, laser pyrolysis, pkant mediated, sol-gel processing, bioassisted synthesis, htdrothermal , plasma spraying, and electrochemical or chemical nano-structural precipitation(Duman et al., 2024).

Some of the most commonly used methods are explained below. These methods have advantages and disadvantages. Particles with optimal properties can be prepared by choosing the most suitable method for the purpose.

1.1.1 Chemical Methods

Currently, the predominant techniques for synthesizing AgNPs still predicated on chemical procedures. Under specific conditions, silver salt precursor (Ag^{+}) is reduced to elemental silver (Ag^{0}) via electron transfer. Nucleation and growth steps occur to produce the final AgNP product. In summary, the concentration of silver in the solution rapidly surpasses the threshold level of supersaturation, resulting in "burst nucleation" and precipitation, which facilitates nucleus formation. In addition to nucleation, a greater input of silver promotes the expansion of nuclei and the production of bigger nanoparticles. The chemical synthesis procedures for AgNPs can provide nanoparticles with minimal aggregation and high output, notwithstanding the elevated production costs and potential hazards.In chemical synthesis processes, the generation of nanoparticles necessitates three reactant components, reducing agents, : a silver salt precursor and a stabilizing agent(Amir et al., 2021; Kim et al., 2004; Nguyen et al., 2023).

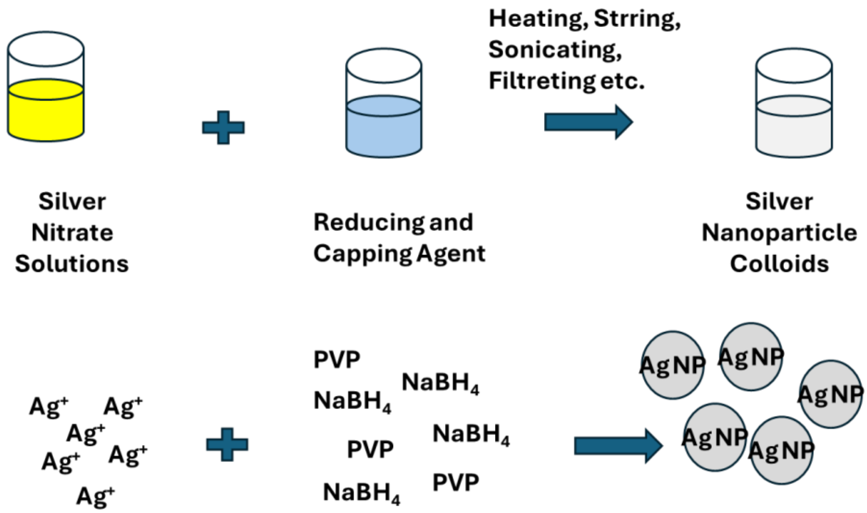

Figure 2: Prosedure of chemical reduction methods for Synthesize Ag NPs

The most commonly used chemical method is the chemical reduction method(Figure 2). In this method, it is usually prepared in hydrophilic meadium, and silver nitrate salt is used as a precursor. The reducing agent is usuallytriethylenetetramine, ethanol ethylene glyco,l ascorbic acid, sodium citrate, dimethyl Formamide sodium borohydrate, chitosan, triethanolamine, phenylhydrazine tetraethylpentaamine, hydrazine hydrat. Capping agents can be used to control shape and size (Naderi-Samani et al., 2023).

1.1.2 Biological Methods

Recent scientific advances indicate that the green synthesis methodology is preferable to chemical and physical synthesis approaches. Silver nanoparticles (AgNPs) generated using biological processes have lately garnered interest due to their ability to mitigate the detrimental impacts of toxic by-products generated during nanoparticle synthesis. Natural extracts, including flora and microbes, are utilized in the biological synthesis process. The biosynthesis approach utilizing biological extracts has garnered significant global interest due to the existence of many metabolites that not only convert Ag+ ions to AgNPs but also encapsulate them, inhibit agglomeration, and diminish toxicity . Furthermore, it is examined that microorganisms-based biosynthesis also produces reductive biomolecules and is environmentally sustainable in natur(Dhaka et al., 2023). General scheme of biological synthesize methods is given at Figure 3.

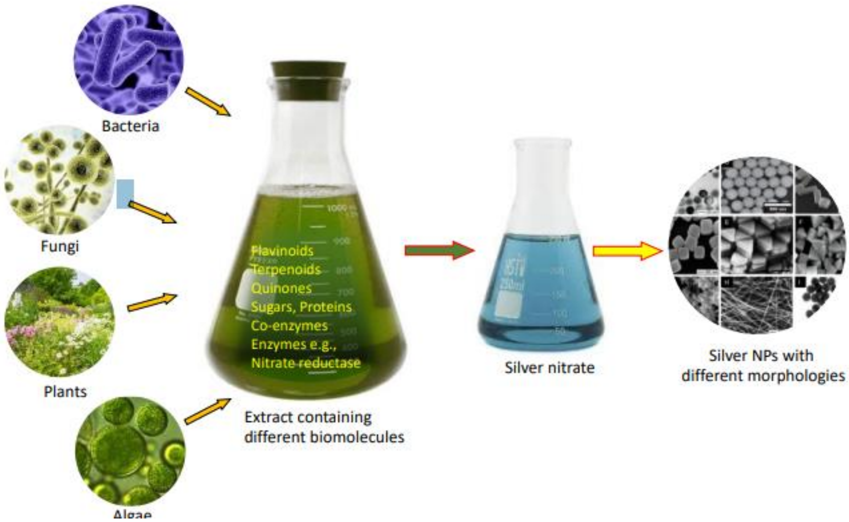

Figure 3: Diffrent reducing reagent sourece of for preperaton silver nanoparticles(Almatroudi, 2024).

The synthesis of silver nanoparticles using biological reagents is increasingly common in the literature. This is because it aligns with the green chemistry approach and the source is obtained from nature. Particles prepared with biological materials are always more preferable for biomedical applications. Bacteria, fungi, plant extracts, enzymes, etc. can be used as biological reagents(Dhaka et al., 2023; Khatun et al., 2023; Singh et al., 2017; Zhao et al., 2018).

1.1.3 Physical Methods

Evaporation-condensation and laser ablation are the key physical methodologies. Laser ablation, , evaporation irradiation condensation, and lithography are the principal physical techniques for synthesizing AgNPs(Nguyen et al., 2023). Figure 4 indicates scheme of most popular physical methods. The lack of solvent contamination in the fabricated thin films and the uniform distribution of nanoparticles are benefits of physical synthesis methods over chemical ones(Iravani et al., 2014).

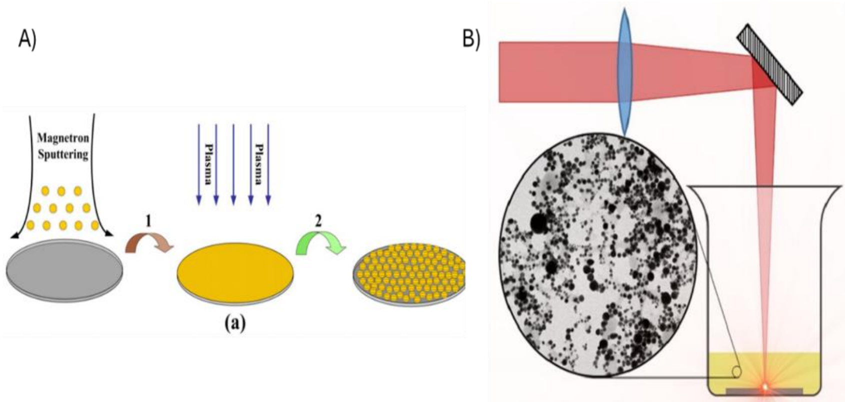

Figure 4: Scheme of A) plasma-induced technique(Shi et al., 2015), and B) laser ablation technique (Díaz-Núñez et al., 2017), for silver nanoparticle preparation

Pysical Methods are identified as an effective alternative to laborious chemical methods. Moreover, the resultant nanoparticles exhibits resistance to aggregation and possessed a restricted size distribution. The potential benefits of physical production methods include speed, the absence of hazardous reagents, and the use of radiation as a reducing agent. Physical approaches have drawbacks such as solvent contamination, low yield, uneven distribution, and elevated energy usage(Almatroudi, 2020b).

1.2 Biomedical Applications

Silver nanoparticles (AgNPs) possess a variety of physicochemical and biological attributes, such as exceptional surface plasmon resonance, elevated surface-to-volume ratio, facile conjugation, magnetic targeting, wound healing efficacy, gene and drug transport capabilities, bio-composite synthesis, and toxicity. Their enhanced antibacterial properties compared to bulk silver and silver ions can be ascribed to their size. Additional attributes, like silver nanostructures and crystallographic patterns, also affect their efficacy against bacteria. Silver nanoparticles (AgNPs) have attracted interest in the biomedical field owing to their luminescent properties, which have been investigated for their effects on fluorescent drug detection. They are frequently utilized in vaccine adjuvants, antidiabetic medications, oncological treatments, biosensing applications, and in the healing of wounds and bones. The luminescent properties of AgNPs have garnered attention in recent studies(Almatroudi, 2020b; Meher et al., 2024a).

1.2.1 Tissue Engineering

Tissue engineering and regenerative medicine (TERM) solutions are increasingly favored due to the obstacles associated with tissue and organ transplantation, including restricted donor availability, immunosuppression, and inadequate success rates. This interdisciplinary domain integrates biological, engineering sciences , and material to fabricate artificial constructs that replicate organic tissues and organs. Nanoparticles (NPs) are utilized to fabricate three-dimensional scaffolding for cells, offering precise control over their characteristics and regulated release of bioactive substances. Nanoparticles mitigate limitations such as poor solubility, unstable bioactivity, and brief circulation half-life of bioactive compounds, rendering them ideal candidates for the distribution and monitoring of bioactive agents. This method facilitates improved oversight and regulation of cellular functions, hence augmenting the efficacy of transplantation(Fathi-Achachelouei et al., 2019).

Anushikaa and co-workers incorporate osteoinductive and antibacterial cue-loaded hydrogels with silver nanoparticles into 3D-printed titanium scaffolds. 3D-Ti scaffolds were fabricated via direct metal laser sintering and infused with a gelatin (Gel) hydrogel incorporating strontium-doped silver nanoparticles (Sr-Ag NPs). The 3D-Ti/Gel/Sr-Ag NPs scaffolds exhibited superior physicochemical properties attributable to Gel and Sr-Ag NPs. Accrding to results They exhibited biocompatibility with mMSCs, demonstrated osteoinductive and osteoconductive characteristics, and had significant antibacterial efficacy against both gram-negative and gram-positive pathogens. The biomaterials offered complementary benefits in bone formation and antibacterial effectiveness(Anushikaa et al., 2024). In another study, Abdelmoneim an co-workers show that alpha lipoic acid-capped AgNPs can efficiently suppress bacteria in novel bone regeneration materials. A dosage of 100 µg/g can effectively suppress bacteria without adversely affecting bone cells. The research demonstrated that the incorporation of AgNPs into bone grafts enhanced osteoblast growth in vitro, without inducing inflammation or negative effects in vivo. The two materials, nanosilver bone graft and nanosilver bone graft combined with hydrogel, have dual roles for infection prevention and bone regeneration(Abdelmoneim et al., 2024).

Vishnevetskii an co-workers have developed potential regenerative medicine which include silver nanoparticle (cysteine-silver gels) to in regenerative medicine but this nonostructure was ineffcient. For this reason, they have to improve this nanostructre. have addressed the problem of inadequate mechanical characteristics in cysteine-silver gels (CSG) by using biocompatible polymers such as polyvinyl alcohol (PVA), polyvinyl pyrrolidone (PVP), and polyethylene glycol (PEG). These polymers exhibit compatibility with cysteine-silver sol (CSS), resulting in the formation of gels with sodium sulfate. PVA-based gels have superior strength and stability owing to their capacity to establish hydrogen bonds with CSS particles.

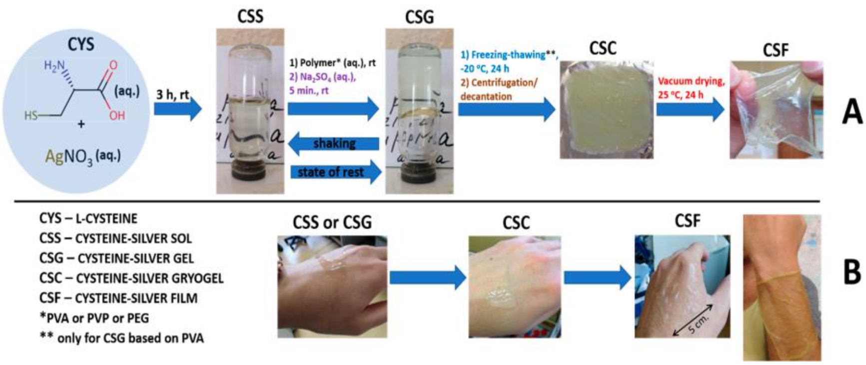

Figure 5: Figure from Vishnevetskii an co-workers studies A) The methodology for the manufacture of gels and films. (B) Abbreviations and the properties of the materials acquired in relation to their activity on the skin

These straightforward operations yield porous films with a stable CSS particle structure, providing excellent mechanical, swelling, and adhesion qualities to skin. In vitro research indicate that gels can modestly stimulate the proliferation of normal human fibroblast cells, implying possible applications in regenerative medicine. Figure 5 shows the wound healing that occurs when the prepared nanostructure is used. (Vishnevetskii et al., 2023).

1.2.2 Anticancer and Antitumor Activity

Cancer is among the most fatal diseases now impacting human health. Research indicates that around one in three individuals may receive a cancer diagnosis at some stage in their lives. This can manifest in various forms depending on its origin, including thyroid, prostate, bladder, kidney, pancreatic, breast, melanoma, all types of leukemia, oral cancer, and colorectal cancer, among others. Cancerous cells proliferate rapidly, forming tumors that invade healthy tissues and disseminate throughout the body. malignant cells proliferate swiftly to produce additional malignant cells. According to recent research, the use of silver particles in the field of anti-tumor and ancient cancer studies has become widespread(Meher et al., 2024b).

Dahran and co-worker focused on anticancer activity of silver nanoparticles.Silver nanoparticles (Ag NPs) have been shown to stimulate autophagy in cancer cells, an essential cellular breakdown mechanism that facilitates cell death. Their research demonstrates that Ag NPs stimulate the PtdIns3K signaling pathway, leading to increased autophagosome formation, proper cargo breakdown, and preservation of lysosomal function. Autophagy facilitates cell survival, while the inhibition of autophagy using pharmacological inhibitors or ATG5 siRNA amplifies the cytotoxic effects of Ag NPs on cancer cells. Their research indicated that wortmannin, a commonly utilized autophagy inhibitor, markedly amplified the anticancer efficacy of Ag NPs in the B16 murine melanoma cell model(Lin et al., 2014).

Dahran and colleagues' study suggests that the potential anticancer properties of vincamine loaded in silver nanoparticles (VCN-AgNPs) were assessed in mice with Ehrlich solid carcinoma (ESC). Vincamine, a monoterpenoid indole alkaloid possessing vasodilatory effects, is derived from the leaves of Vinca minor. VCN-AgNPs decreased angiogenesis by reducing VEGF levels in the tumor's cells, resulting in apoptosis. Figure 6 Furthermore, histological analyses demonstrated that VCN-AgNPs inhibited the advancement of Ehrlich carcinoma and prompted the development of clusters of necrotic and fragmented tumor cells. VCN-AgNPs have cytotoxic and genotoxic effects on ESC due to their prooxidant, pro-apoptotic, pro-inflammatory, and antiangiogenic properties. Figure 6 indicateh that anticancer effect of silver nanoparticles which are combined witth drug.

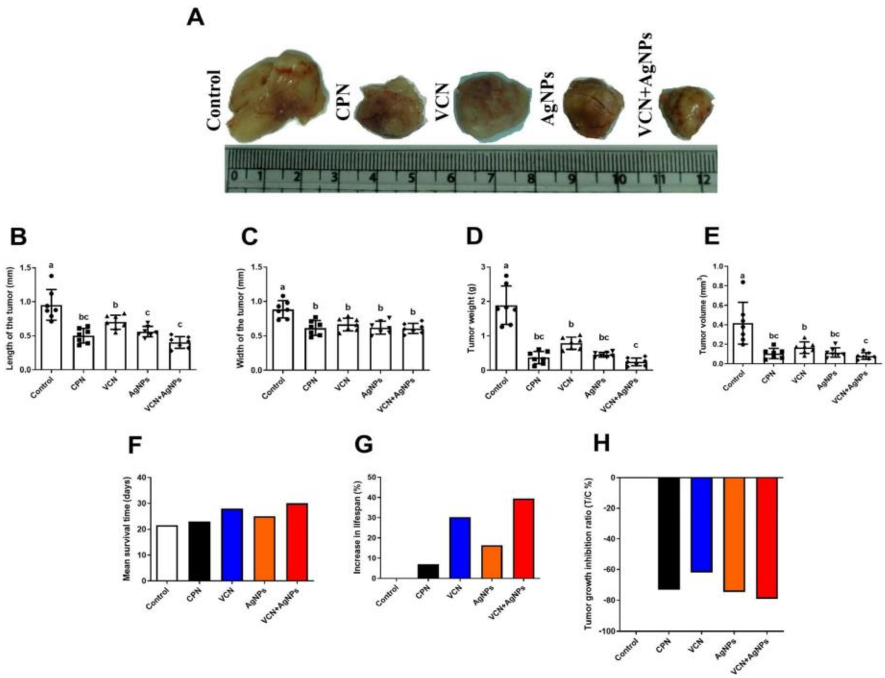

Figure 6: Efficacy of CPN, VCN, AgNP, and VCN-AgNP treatment on tumor size (A) induced Ehrlich solid tumors (ESTs) in various cohorts, (B) length, (C) width, (D) tumor weight, (E) tumor volume, (F) mean survival duration, (G) percentage of IMLS, and (H) tumor growth inhibition index (T/C %) in mice with Ehrlich solid carcinoma(Dahran et al., 2024).

The amalgamation of VCN-AgNPs proved to be more efficacious as well as less hazardous than chemically manufactured AgNPs, as by an extension in the lifetime of subjects and an elevated overall tumor inhibition index (Dahran et al., 2024).

1.2.3 Antibacterial, Antiviral and Antifungal Activity

Silver nanoparticles are highly significant due to their prospective use as antimicrobial, antiviral and antifungal agents in nanomedicine, groundwater remediation, surgical mask production, wound dressing creation, and textile materials for military use. Speciallt there ara too many product which are developed by using silver nanoparticle for their uniq antibacterial properties. The primary benefit of silver nanoparticles over metallic silver or its salts is the controlled release of silver ions, which possess a prolonged antibacterial effect. The likelihood of microorganisms developing sensitivity to AgNPs is significantly lower compared to the effects of antibiotics. The swift proliferation of drug-resistant disease strains necessitates the creation of novel antibacterial composites . Antibiotics that were previously effective against pathogenic bacteria are now predominantly ineffective . Bacterial resistance diminishes the efficacy of frequently prescribed antibiotics, potentially facilitating the propagation of diseases in humans as well as animals(Abbas et al., 2024; Burdușel et al., 2018; Lin et al., 2014).

Baelga and co- workersdeveloped a highly antiviral agent by attaching silver nanoparticles to polyethyleneimine (PEI) fibers via electrostatic interaction. They demonstrated that this silver nanoparticle-containing (Ag-Nps-PEI) substrate was more effective against the SARS-CoV-2 virus than a PEI-only substrate(Baselga et al., 2022). In another study. Makhlof and co workers investigated the antiviral effects of silver nanoparticles prepared with plant extracts using the green chemistry method and showed that these nanoparticles have antiviral effects against adenovirus(Makhlof et al., n.d.).

Wtih same aim Hashem and co-worker investagetd antifungal effect of silver nanoparticle.This study reports the successful biosynthesis of silver nanoparticles (AgNPs) using a cell-free extract (CFE) from Bacillus thuringiensis MAE 6 via a green and eco-friendly method. AgNPs demonstrated enhanced antifungal efficacy against four *Aspergillus* species responsible for Aspergillosis, specifically *Aspergillus niger*, *A. terreus*, *A. flavus*, and *A. fumigatus*. Compared to silver nanopartcle, of AgNPs, Nystatin (NS), and AgNO3had low antifungalactivity(Figure 7). The biosynthesized AgNPs utilizing a CFE of B. thuringiensis demonstrate potential as an antifungal agent against *Aspergillus* species(Hashem et al., 2022).

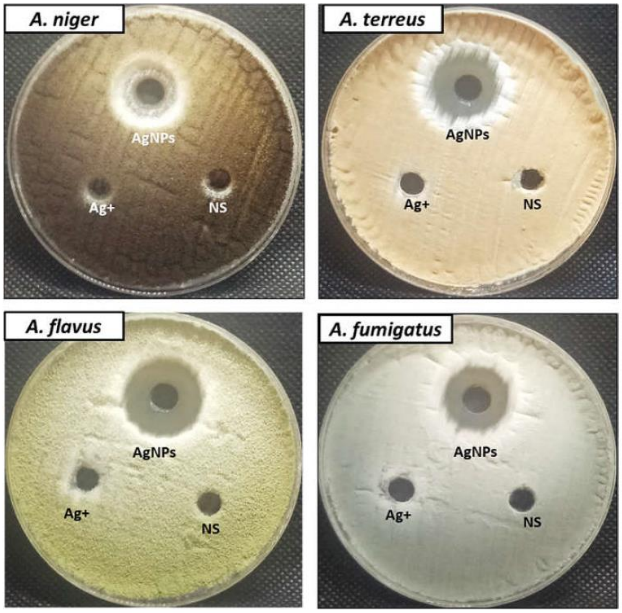

Figure 7: The antifungal activity of AgNPs, AgNO3, and Nystatin (NS) at a concentration of 500 µg/mL was assessed against *A. niger, A. terreus, A. flavus*, and *A. fumigatus* using the agar well diffusion method.

Das and colleagues developed an environmentally friendly method for the synthesis of pure, safe, and stable silver nanoparticles (AgNPs) utilizing *Trema orientalis (L.)* leaf extract as both a reducing and stabilizing agent, and assessed its antibacterial activity. The well diffusion method exhibited the antibacterial efficacy of AgNPs against Staphylococcus aureus. This study presents a more sustainable and efficient approach to bacterial treatment(Das et al., 2025). Jamil and colleagues synthesized silver nanoparticles (AgNPs) through a green synthesis method utilizing Aloe 1eurentinorum plant extract. This study demonstrates that the antimicrobial activity of the synthesized AgNPs is more effective against Gram-negative bacteria compared to Gram-positive bacteria, particularly highlighting their efficacy against *Escherichia coli*(Jamil et al., 2024).

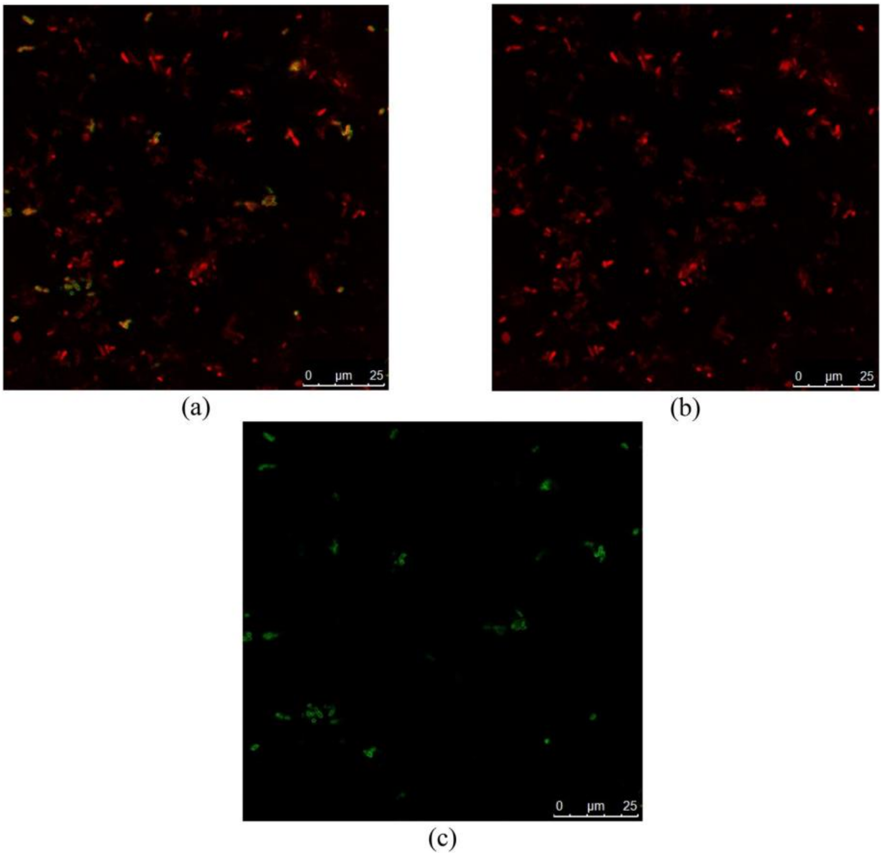

Figure 8: Confocal microscopy analysis of bacterial-treated cells, categorized as (a) merged, (b) dead, and (c) alive cells (Elwakil et al., 2024).

AgNPs@C were synthesized efficiently using an electrical arc generated by a single spark unit, which effectively ionized the dielectric medium (deionized water) by applying a strong electric field within the silver and carbon electrodes. The synthesized nanoparticles were evaluated for their antibacterial effect against Pseudomonas aeruginosa. compared to Ceftazidime, a commonly utilized antibiotic for P. aeruginosa infections. The AgNPs@C shell demonstrated enhanced activity. The observed activity was corroborated by confocal microscopy, which revealed an increased red region indicative of dead cells, correlating with a presence of AgNPs@C. The antibacterial efficacy of the synthesized nanoparticles was evaluated against Pseudomonas aeruginosa in comparison to the commonly utilized antibiotic Ceftazidime. The

AgNPs@C shell demonstrated enhanced activity compared to the antibiotic employed. Confocal microscope images proved this stiutation. (Elwakil et al., 2024).

2. CONCLUSION

In this study, current information is given regarding the preparation of silver particles and their use for biomedical purposes. Silver nanoparticles are currently prepared using various approaches. The most classic of these is chemical reduction, while physical methods are less commonly used. However, as frequently mentioned in the study, the Green synthesis approach has been widely used for the preparation of silver nanoparticles for the last ten years. In this approach, plant extracts are used as reducing agents instead of chemicals that are hazardous to nature. With this approach, both natüre friendly silver nanoparticles are obtained and waste plants are evaluated for this purpose. Studies show that the use of silver nanoparticles for biomedical purposes is a very accurate hypothesis. Because, in addition to their antibacterial effects, their anti-cancer, anti-fungal and anti-viral activity make these particles unique in biomedical applications. Silver Nanoparticles are also widely used in the field of tissue engineering and are aimed to be used in the construction of artificial tissues thanks to their antimicrobial properties mentioned above and the increased stability of the nanocomposites they contain. Current studies show that Silver Nanoparticles will be used in Biomedical applications in the coming years and will play a leading role in this field.

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CHAPTER 13

INTEGRATED NUTRIENT MANAGEMENT IN POMEGRANATE (*Punica granatum* L.) PRODUCTION ENHANCING YIELD, FRUIT QUALITY, AND OVERCOMING PHYSIOLOGICAL DISORDERS

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1. INTRODUCTION

Pomegranate (*Punica granatum* L.), belonging to the Puniceae family, is a globally significant fruit species widely consumed fresh and in processed forms like juice, wine, molasses, and jam. Its high antioxidant content and nutritional value make it particularly prominent in modern healthy eating trends (Figure 1), (Levin, 2006; Mphahlele et al., 2016). Global pomegranate production has been steadily increasing, especially in the Mediterranean Basin (FAOSTAT, 2023). Despite its considerable commercial potential, pomegranate cultivation faces challenges such as low yield, fruit quality issues, and various physiological disorders, notably fruit cracking (Srivastava et al., 2008; Singh et al., 2020). These issues adversely affect market value and grower profitability.

Plant nutrition is a critical factor for achieving optimal growth, high yield, and superior fruit quality. A balanced supply of macro and micro-nutrients is vital for healthy physiological processes within the plant (Marschner, 2012). Soil properties, climatic conditions, and cultivar variations directly influence nutrient availability and efficacy. This review aims to comprehensively examine soil and foliar fertilization strategies, based on current scientific literature, to enhance yield, fruit quality, and overall plant performance, while minimizing physiological disorders in pomegranate production.

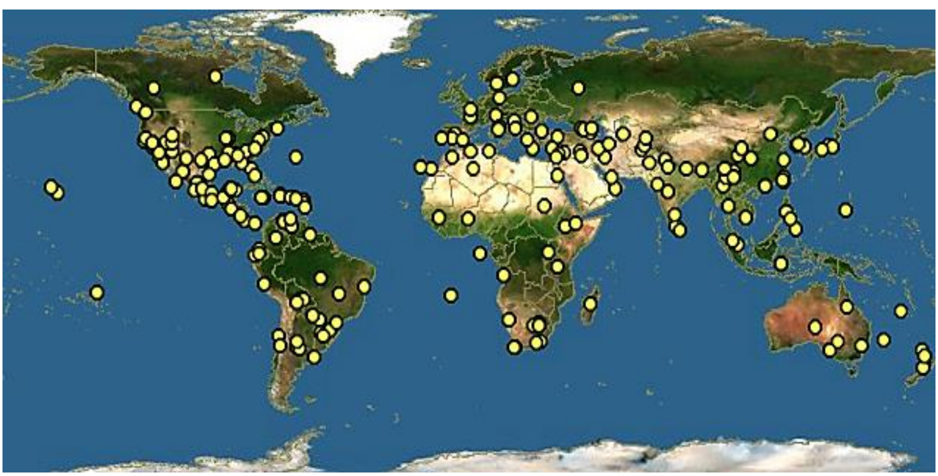

Figure 1: Map of world pomegranate production areas

2. NUTRIENT REQUIREMENTS AND FUNCTIONS OF POMEGRANATE

For healthy development, high yield, and quality, a balanced uptake of essential macro, meso and micro-nutrients is crucial for pomegranate trees (Figure 2).

Figure 2: Pomegranate leaf, flower and fruit images

2.1. Macronutrients

- **Nitrogen (N):** A primary driver of vegetative growth, nitrogen is a fundamental component of chlorophyll, proteins, and nucleic acids. Adequate N promotes robust shoot development and increases photosynthetic activity, positively influencing fruit set and size. However, excessive or untimely N application can reduce fruit quality (sugar-acid balance, coloration) and increase cracking susceptibility (Naik et al., 2024; Alva et al., 2005).
- **Phosphorus (P):** Indispensable for energy transfer, root development, flowering, and fruit formation (Hopkins and Hüner, 2009). Sufficient P supports strong root systems, optimizing water and nutrient uptake, and encourages flowering (Khan et al., 2023).
- **Potassium (K):** A critical element regulating osmotic balance, turgor, and water use efficiency. It activates enzymes, supports protein synthesis, and is vital for photosynthesis and sugar translocation. Adequate K directly improves fruit size, soluble solids content (TSS), coloration, and extends shelf life (Mengel and Kirkby, 2001; Tehranifar et al., 2010).

2.2. Mesonutrients

- **Calcium (Ca):** Plays a central role in maintaining the structural integrity and elasticity of plant cell walls, influencing fruit firmness, storage life, and critically, resistance to cracking (Saure, 2005; Sheikh and Manjula, 2012; Davarpanah et al., 2018). Due to its limited mobility within the plant, particularly to fruits, foliar applications are often essential even when soil Ca levels are adequate.
- **Magnesium (Mg):** The central atom of the chlorophyll molecule, making it essential for photosynthesis. It also plays a role in enzyme activation and protein synthesis (Marschner, 2012).
- **Sulfur (S):** A component of amino acids (cysteine, methionine) and proteins, and contributes to the synthesis of compounds that enhance plant stress resistance (Hopkins and Hüner, 2009).

2.3. Micronutrients

- **Boron (B):** A critical micronutrient essential for cell wall synthesis, sugar transport, pollen germination, and pollen tube growth (Blevins and Lukaszewski, 1998; Vera-Maldonado et al., 2024). In pomegranate, adequate B is crucial for successful flowering, fruit set, and interacts synergistically with calcium to reduce fruit cracking.
- **Zinc (Zn):** Involved in auxin (growth hormone) synthesis and activates numerous enzymes. Deficiency leads to stunted growth and reduced yield (Marschner, 2012).
- **Iron (Fe):** A component of enzymes involved in chlorophyll synthesis, photosynthesis, and respiration. Deficiency causes interveinal chlorosis, especially in calcareous soils (Hopkins and Hüner, 2009).
- **Manganese (Mn):** Activates enzymes involved in photosynthesis (water splitting), chlorophyll synthesis, and carbohydrate metabolism (Marschner, 2012).
- **Copper (Cu):** A component of enzymes involved in photosynthesis and respiration, contributing to lignification and cell wall formation (Marschner, 2012).

- **Molybdenum (Mo):** Essential for nitrogen metabolism, particularly the nitrate reductase enzyme (Marschner, 2012).

3. FERTILIZATION APPLICATION METHODS

Nutrient supply to plants is primarily achieved through soil and foliar applications, each with distinct advantages and disadvantages.

3.1. Soil Application

The most traditional method, involving direct incorporation into the soil or surface application followed by irrigation or rainfall.

- **Advantages:** Provides a long-term nutrient supply, allows for high quantities of nutrients to be applied, and promotes strong, deep root development (Brady and Weil, 2008).
- **Disadvantages:** Nutrient use efficiency can be low due to losses from fixation, leaching, or volatilization. Efficacy is highly dependent on soil pH, organic matter content, and texture (Marschner, 2012; Alloway, 2008).

3.2. Foliar Application

Nutrients are sprayed directly onto the leaf surface and absorbed through stomata or the cuticle, entering plant metabolism (Fernández and Brown, 2013).

- **Advantages:** Rapid nutrient uptake and quick correction of deficiencies, overcomes soil-related limitations (e.g., high pH, calcareous soils) by bypassing root uptake, and allows for targeted nutrient supply during critical phenological stages (Fernández and Brown, 2013; El-Salhy et al., 2022).
- **Dezavantajları:** Risk of phytotoxicity at high concentrations, limited nutrient absorption capacity makes it unsuitable as a sole source for macronutrients (Fernández and Brown, 2013).

3.3. Fertigation (Fertilization with Irrigation)

Nutrients are delivered to the root zone via irrigation water, widely used in modern pomegranate cultivation with drip irrigation systems.

- **Advantages:** High nutrient use efficiency due to precise delivery to the active root zone, controlled nutrient dosage according to plant phenology, reduced labor costs, and improved nutrient availability even in challenging soils (Marschner, 2012).

- **Dezavantajları:** High initial setup cost, requires technical expertise for proper program management, and potential for clogging irrigation emitters if not managed correctly.

4. FERTILIZATION STRATEGIES FOR ENHANCING YIELD AND PERFORMANCE

Achieving high and quality pomegranate yields necessitates a balanced, sufficient, and timely supply of nutrients. Fertilization programs should be tailored based on soil nutrient status (soil analysis), tree age, cultivar characteristics, climatic conditions, and target yield (Marschner, 2012).

- **Balanced Nutrition:** Maintaining specific ratios among nutrient elements is vital for healthy growth. Deficiency or excess of one element can negatively impact the uptake and utilization of others (Marschner, 2012). Regular soil and leaf analyses are crucial for identifying current nutrient status and adjusting fertilization programs (Alva et al., 2005).
- **Recommendations for Key Elements:**
  - ✓ **Nitrogen (N):** Annually 50-100 g/tree for young trees, 150-300 g/tree for bearing trees, applied in 2-3 split doses starting before vegetative growth in spring and after fruit set (Naik et al., 2024).
  - ✓ **Phosphorus (P):** Annually 30-60 g/tree P_2O_5; applied in late winter or early spring near the root zone (Khan et al., 2023).
  - ✓ **Potassium (K):** Annually 150-400 g/tree K_2O for bearing trees; split applications from spring to late summer, especially during fruit growth and ripening (Tehranifar et al., 2010; Kumar et al., 2020).
  - ✓ **Calcium (Ca):** 3-5 foliar applications of 0.5%-1.0% calcium nitrate or calcium chloride solution, starting after fruit set (e.g., nut size) at 2–3-week intervals until 3-4 weeks before harvest (Sheikh and Manjula, 2012; Davarpanah et al., 2018: Singh et al., 2020).
  - ✓ **Boron (B):** 1-2 g L^{-1} boric acid or sodium borate foliar applications before flowering (pink bud stage) and immediately after fruit set enhance yield and quality (Maity et al., 2006).

- ✓ **Zinc (Zn):** 0.05%-0.1% zinc sulfate or chelate foliar applications at the onset of vegetative growth or before flowering (Mirdehghan and Rahemi, 2007).
- ✓ **Iron (Fe):** In calcareous soils, Fe-chelates (e.g., Fe-EDDHA) can be soil applied, or 0.2%-0.5% iron sulfate foliar applied when chlorosis symptoms appear or during early vegetative growth (Gajbhiye et al., 2014).

- **Phenological Stage-Specific Nutrition:** Nutrient demands vary throughout the growth cycle. Programs should be optimized for dormant period, pre-flowering, fruit set, and fruit development/ripening stages.

5. MINIMIZING PHYSICAL DISORDERS (ESPECIALLY FRUIT CRACKING)

Fruit cracking is the most prevalent and detrimental physiological disorder in pomegranate, resulting from the fruit peel's inability to withstand increasing internal pressure during growth (Yılmaz and Özgüven, 2003).

5.1. Causes of Fruit Cracking

Cracking is a complex interaction of genetic predisposition, environmental factors, and nutritional imbalances (Singh et al., 2020). Key environmental triggers include erratic irrigation (sudden heavy rainfall or irrigation after prolonged drought) (Chandra et al., 2018), low atmospheric humidity, and high temperatures, which reduce peel elasticity (Mirdehghan and Rahemi, 2007).

5.2. Role of Calcium and Boron in Cracking Control

Calcium and boron are crucial for fruit peel integrity and elasticity, often acting synergistically to enhance cracking resistance:

- **Calcium (Ca):** Strengthens cell walls by forming cross-linkages with pectic substances, imparting elasticity and resistance to internal pressure as the fruit expands (Saure, 2005). Due to limited transport to fruits via the xylem, foliar applications are essential for effective cracking control.

- **Boron (B):** Plays a critical role in cross-linking pectin in cell walls, enhancing peel flexibility and strength, and facilitating sugar transport (Vera-Maldonado et al., 2024; Hu and Brown, 1994).
- **Application Recommendations:** Foliar applications of calcium (e.g., 0.5%-1.0% calcium nitrate/chloride) and boron (e.g., 1-2 g L^{-1} boric acid/sodium borate) are highly effective in reducing cracking risk when applied during fruit development, starting from fruit set (Yılmaz and Özgüven, 2003; Maity et al., 2006).

5.3. Role of Other Factors

Balanced potassium application is important, as excessive K can sometimes exacerbate cracking. Most critically, **consistent and adequate irrigation management** during fruit development, avoiding sudden fluctuations in soil moisture, is paramount for preventing cracking (Chandra et al., 2018).

6. CULTIVAR, CLIMATE AND SOIL INTERACTIONS

Pomegranate nutrient response and the efficacy of fertilization strategies are significantly influenced by cultivar genetics, climatic conditions, and soil properties.

- **Cultivar Effect:** Different pomegranate cultivars exhibit variations in nutrient uptake efficiency, growth habits, yield potential, quality attributes, and susceptibility to physiological disorders (Holland et al., 2009). Fertilization programs should be adapted to cultivar-specific needs.
- **Climate Effect:** Temperature, rainfall, humidity, and light intensity directly or indirectly affect nutrient uptake, translocation, and metabolic activity. Erratic rainfall patterns and low atmospheric humidity, in particular, increase fruit cracking risk (Chandra et al., 2018).
- **Soil Effect:** Soil pH, organic matter content, texture, and drainage critically determine nutrient availability. High pH and calcareous soils, common in Mediterranean climates, can limit the availability of micronutrients like Fe, Zn, and B, necessitating the use of chelated fertilizers or foliar applications (Alloway, 2008). Organic matter enhances nutrient retention and availability (Brady and Weil, 2008).

7. CONCLUSION AND FUTURE RESEARCH DIRECTIONS

Achieving optimal yield, superior fruit quality, and minimizing physiological disorders in pomegranate cultivation is contingent upon the implementation of integrated nutrient management strategies. A balanced supply of macro, meso, and micro-nutrients (especially Ca and B) at the right time and through appropriate methods directly impacts plant health and fruit integrity. Foliar fertilization provides an effective supplement when soil conditions are limiting or during periods of high nutrient demand, while fertigation enhances nutrient use efficiency. Fertilization programs must be tailored to specific cultivar, climatic, and soil conditions.

Future research should focus on:

- ❖ **Cultivar-Specific Nutrient Needs:** More detailed investigations into the unique nutrient uptake efficiencies and optimal concentrations for different pomegranate cultivars, including local and underutilized varieties.
- ❖ **Micronutrient Mechanisms:** Deeper understanding of the precise mechanisms and synergistic interactions of micronutrients (e.g., Zn, Fe, Mn) on fruit quality and physiological disorders beyond Ca and B.
- ❖ **Fertigation Optimization:** Long-term studies on optimizing nutrient ratios and timing for water and nutrient application through fertigation systems across different phenological stages.
- ❖ **Stress Tolerance and Nutrition:** Exploring how abiotic stresses (drought, salinity, high temperature) affect nutrient uptake and utilization in pomegranate, and how nutrient management can enhance plant resistance to these stresses.
- ❖ **Bioregulators and Nutrient Interactions:** Further research on the interactions between plant growth regulators and nutrient elements on pomegranate yield, quality, and stress responses.
- ❖ **Environmental Sustainability:** Promoting studies on the environmental impacts of fertilization (soil and water pollution) and developing innovative, eco-friendly fertilization technologies to improve nutrient use efficiency.

These research directions will contribute to developing science-based solutions for overcoming challenges in pomegranate cultivation, ensuring sustainable, productive, and high-quality output in the global market.

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CHAPTER 14

SMART MECHATRONIC APPLICATIONS IN AGRICULTURE: PRESENT INSIGHTS AND PERSPECTIVES

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1. INTRODUCTION

Agricultural food production, directly proportional to the world's rapidly growing population, has been made possible by the mechanization that has surged in the last century. However, because population growth continues to increase, research on smart and efficient systems in agriculture continues unabated. Mobile agricultural machinery in the last century was groundbreaking in the 1900s, but today, current technologies such as autonomous robots, unmanned aerial vehicles, image processing, and machine learning are also being applied to agriculture. Furthermore, due to the unpredictability and variability of external factors in agriculture, production processes require specialized approaches to improve. This is where mechatronic systems used during product production come into play. What are these mechatronic systems? Examples include smart automation systems, artificial intelligence technologies, cloud systems, agricultural robots, and self-powered sensors. Unpredictable and variable conditions in agriculture can be eliminated and adapted to dynamic conditions with computer-aided learning technologies.Advanced technologies such as autonomous robots, unmanned aerial vehicles (UAVs), image processing systems, artificial intelligence, and machine learning are now being actively implemented in agriculture. These technologies enable production processes to be carried out more precisely, quickly, and efficiently, while also having the potential to minimize human errors. For these and similar reasons, the application of unmanned vehicles, smart systems, and robotic platforms in agriculture has become a global trend in recent years [1]. This study examines the role, importance, contribution, and future prospects of mechatronic applications in smart agriculture.

2. SMART MECHATRONIC SYSTEMS IN AGRICULTURE

Smart mechatronic systems are currently used in many different fields, particularly in the automotive, education, aviation, healthcare, the service sector, and agriculture.

The primary purpose of mechatronic systems is to improve and simplify human life and ensure environmental sustainability.

This study covers smart mechatronic systems in agriculture only.

Agriculture has a long history of initially relying entirely on human labor. Later, animal power emerged with mechanical advancements such as diesel/steam-powered tractors and hydrostatically driven mechanical devices requiring control [2].

Today, this mechanization is no longer sufficient, and autonomous mechatronic systems that mimic human intelligence and add intelligence to these systems are gaining importance. In this context, we examine systems that can generate their own energy, predict weather and crop yields, utilize unmanned aerial vehicles, and utilize sensors to monitor growth and pests through image processing. Mechatronic systems, the intersection of computer, mechanical, and electrical-electronic sciences, have become indispensable to the agricultural sector.

The main areas where smart mechatronic technologies stand out in agriculture and their contributions to these areas are given in Table 1.

Table 1. Some uses and purposes of smart technologies in agriculture [3]

1. Autonomous farm machinery	They perform tasks such as tillage, planting, sowing, weeding, fertilizing, spraying and irrigation without human intervention.
2. Drone-supported farming	They are used in processes such as remote field monitoring, mapping, plant height determination, irrigation adequacy, pest and weed monitoring, spraying, and product classification.
3. Smart dairy farming	Mechatronic systems are used in processes such as animal monitoring, feed mixing, automatic milking, and daily nutrition analysis.
4. IoT-enabled livestock monitoring	Data from sensors attached to the animals is used to analyze their health status (such as pulse, temperature, and rumination). Precautions can be taken to address potential health issues.
5. Smart poultry farming	Smart sensors are used in processes such as automatic egg collection and controlling environmental conditions such as temperature, humidity and light in poultry houses.
6. Smart greenhouses	In addition to controlling parameters such as humidity, temperature, light amount, and pH in

	greenhouses, the energy required for greenhouse automation is met through integrated renewable energy systems.
7. Smart irrigation	Smart irrigation systems, which analyze the water content in the field based on the information received from IoT-based sensors, are also examples of mechatronic systems.
8. Smart warehousing	With smart storage systems, inventory costs are reduced, and spare parts required for machinery and equipment can be ordered automatically.
9. Auto-steering	
10. Smart harvesting machinery	Smart harvesting machines provide operators with better visibility thanks to cameras, while location data can be obtained from the field thanks to the GPS feature, eliminating repetition.
11. Precision farming	Precision agriculture means using all resources without waste. Sustainability is achieved by using field data correctly.
12. Farming productivity	Remote fault detection in machines ensures uninterrupted production and increased profitability.
13. Training requirements	It can be challenging for farmers to keep up with these new technologies, so training processes for farmers are necessary.
14. Employment opportunities	The reduction of human labor in agriculture by mechatronic systems causes unemployment.
15. Land use	Mechatronic systems enable more land to be used for agricultural purposes.
16. Mobile applications	Due to smartphone applications, farmers can access the field remotely and at any time.
17. Blockchain technology	It allows easy tracking of the supply chain.

[3]

2.1 ARTIFICIAL INTELLIGENCE AND MACHINE LEARNING APPLICATIONS

The world population is expected to reach 9 billion by 2050, and it is estimated that global food production will need to increase by 60% to meet this demand [4]. Several challenges must be overcome to meet this food consumption. One of these is the conversion of natural forests to agricultural land, which harms wildlife, and as a result, many plant and animal species are endangered. Another challenge is the unpredictable conditions in agriculture. For example, climate change, soil degradation, or political and economic turmoil disrupt agricultural production, negatively impacting food production. The use of digital technologies emerges as a powerful tool to overcome these challenges. These technologies, which enable prediction, decision-making, and real-time data flow based on available data and information, aim to prevent production disruptions. While research on artificial intelligence dates back decades, the use of artificial intelligence in agriculture is still in its infancy.

AI technologies in agriculture are used throughout the entire food supply chain, from food production, processing, storage, distribution, retail and consumption to food waste recycling. Figure 1 summarizes the areas where artificial intelligence is used in the food supply field.

In short, by fully utilizing artificial intelligence in agriculture, productivity increases, natural resource use decreases/optimizes, and sustainability increases.

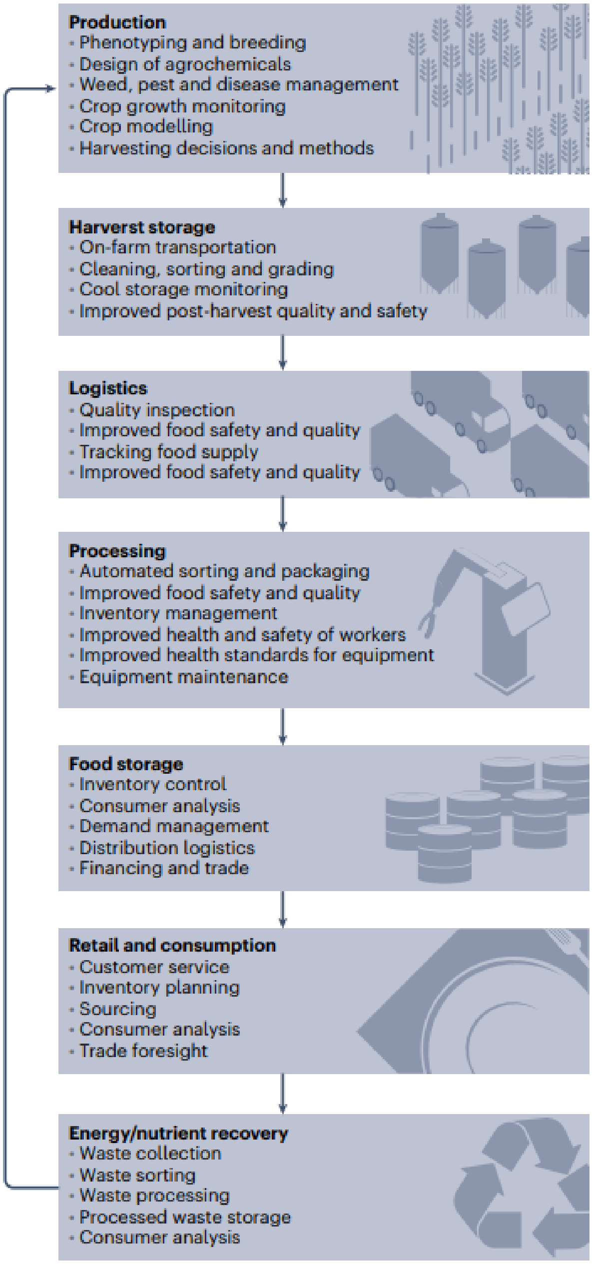

Figure 1. Processes where AI is used throughout the food supply chain in agriculture **[5]**

2.2 AGRICULTURAL ROBOTICS AND AUTONOMOUS VEHICLES APPLICATIONS

The modern world has turned to digital technologies and precision agriculture practices to meet the growing population and food demand. However, this demand cannot be met solely with software and sensors. The effective use of agricultural robots, which can perform difficult tasks by reducing labor and increasing productivity, is seen as a solution to the growing food demand. Advances in software, control, and sensor technology are progressing in parallel with the development of robotic systems. Agricultural robots are currently used for specific tasks, including harvesting, pruning, weeding, crop detection, and sorting. However, agricultural robots must move with greater precision and environmental friendliness than other industrial robots. Due to the conditions of field and greenhouse environments, imprecise robot movements can damage crops and plants. Therefore, precise control and speed are crucial for agricultural robots. For example, a harvesting robot must gently grasp and pick ripe fruit with its end effector without damaging it. While doing so, it must plan its path accurately and avoid collisions, damaging the fruit and trees. This task is more challenging than the task of an industrial robot placing a bolt on an assembly line [6]. To accomplish this challenging task, research in this area is progressing along two main lines. These can be categorized under three headings: precision gripper designs, algorithms enabling robot movement, and high-resolution imaging systems.

In the literature, harvesting robot studies have been conducted on crops such as strawberries [7], cucumbers [8, 9], almonds [10], tomatoes [11,12], citrus [13], mangoes [14,15], and apples [16,17]. Figure 2 shows robots harvesting different fruits. Looking at the robots in the figure, it's clear that each gripper is designed according to the sensitivity and size of the fruit it will harvest. Machine learning algorithms developed during this harvesting process directly impact the robot's performance, allowing it to distinguish between ripe and unripe fruit with high accuracy. Environmental conditions, such as lighting conditions and plant leaf density, also affect robot performance. Continuing research in agricultural robotics is increasing the efficiency, productivity, and other advantages of robots.

a) citrus robot

b) apple robot

c) strawberry robot

d) cucumber robot

e) tomato robot (ridder.com)

Figure 2. Robots used in different fruit harvests [6]

Another advancement in agricultural robots is the improvement of motion algorithms and machine vision systems. Using robots to estimate yield, decide on thinning operations to increase yield, weed detection, and spraying are among the topics of interest and potential for development.

A study in the literature presents the design and results of a four-wheeled weed detection robot. According to the results, a change to the wheel modules will increase the wheel rotation to 180 degrees, allowing the positioning system to operate more reliably and robustly. Research on the four-wheeled weed detection robot continues, focusing on both path tracking and control mechanisms and the development of a planning and management interface [18]. In another study, weeds were removed from a rice field using a robot. The crop quality in the weeded area was compared with the unweeded area. Weeding using the robot was found to have a positive effect on crop yield and size in terms of plant height and weight [19].

Another area where agricultural robots are used is pesticide application. Robots are more reliable in applying these pesticides, which are harmful to human health. Furthermore, they use image processing to optimize the amount of pesticide applied [20].

By combining robotic technologies and artificial intelligence, farmers can analyze fruit growth and quality using images captured from the field. For example, a robot integrated with six RGB cameras was used to estimate yield in an apple orchard. Here, MLP and CNN artificial neural networks were used to process the images. The system's success rate was reported as 82.5% accurate apple detection [21].

Unmanned aerial vehicles, which we might call flying robots, are also used for a variety of purposes in agriculture. Aerial robots that perform tasks such as yield estimation, spraying, mapping, weed detection, and irrigation have been studied in the literature. On very large farms, unmanned aerial vehicles are used to monitor crop growth or disease, as these processes are too difficult to perform manually in large areas. Using UAVs, images can be captured during close-range flights, and these images can be analyzed to identify yields or diseases [22].

3. FUTURE PERSPECTIVE AND CONCLUSION

Roboticization in agriculture will replace human labor for crop planting and harvesting. This will reduce costs and increase productivity through reduced labor. Data received from sensors in the fields, aerial robots, or mobile robot sensors will be processed for plant monitoring, environmental monitoring, and pest monitoring. This data will be evaluated and decision-making processes will be activated. Pesticide and herbicide use will be minimized. During harvest, robots will use specially designed end-processors to ensure harvesting without damaging the crop, and then, if necessary, sorting will be performed based on product quality. Thus, sustainable agricultural practices that reduce labor and increase productivity with robotic systems will become prominent in the future. As data-based decisions are made with artificial intelligence applications in agriculture, resource utilization increases, product yield is predictable, and risk and waste are reduced. Roboticization in agriculture will replace human labor for crop planting and harvesting. This will reduce costs and increase productivity through reduced labor. Data received from sensors in the fields, aerial robots, or mobile robot sensors will be processed for plant monitoring, environmental monitoring, and pest monitoring. This data will be evaluated and decision-making processes will be activated. Pesticide and herbicide use will be minimized. During harvest, robots will use specially designed end-processors to ensure harvesting without damaging the crop, and then, if necessary, sorting will be performed based on product quality. Thus, sustainable agricultural practices that reduce labor and increase productivity with robotic systems will become prominent in the future.

It is anticipated that the use of mechatronic systems in agriculture will reduce product costs. The use of robots will be more preferred in applications that could harm human health (e.g., pesticide applications). Mechatronic systems will operate more efficiently in difficult terrain. Developments in sensors, such as their size and self-powered nature, will directly positively impact roboticization in agriculture.

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CHAPTER 15

MOLECULAR PHYLOGENETICS OF TURKISH *Abies* (PINACEAE) SPECIES BASED ON *matK* GENE REGIONS OF CHLOROPLAST GENOME

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INTRODUCTION

Overview of Pinaceae and *Abies* Genus

The Pinaceae family stands as the largest among the conifers, consisting of nearly 260 species primarily distributed across temperate zones of the Northern Hemisphere. Fossil evidence traces the family's origins back to the Cretaceous period. Species in this family are economically valuable, widely used for timber, pulp production, oils, and resins, and some are also cultivated ornamentally. Among them, the genus *Abies*—the second largest in the family—comprises over 51 species native to North and Central America, Europe, North Africa, and large parts of Asia, including China, the Himalayas, and Taiwan (Esteban et al., 2010; Liu, 1971).

Abies species typically favor mountainous and temperate regions. Although the wood is not ideal for general construction, it is used extensively for pulp, plywood, and indoor construction due to its resistance to pests and decay. Species such as *Abies nordmanniana*, *A. procera*, *A. fraseri*, and *A. balsamea* are among the most preferred Christmas trees due to their aromatic foliage and needle retention. Additionally, species like *A. koreana* and *A. fraseri* are frequently used in landscaping for their distinctive cones (www.conifers.org, 2010) (see Figure 1).

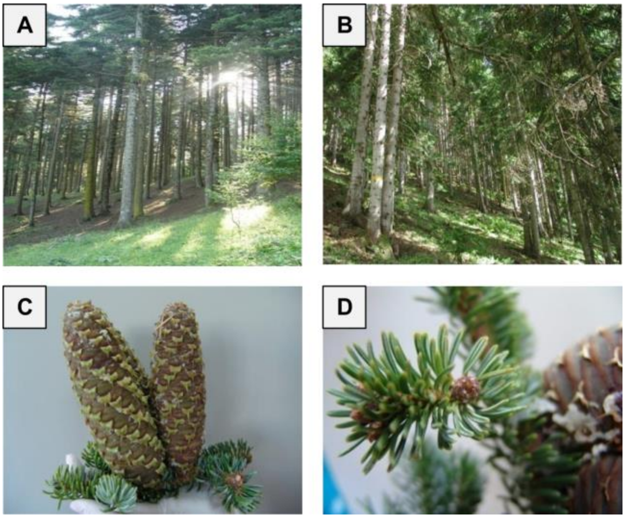

Figure 1. General appearance of Fir and some of its features A. General appearance *(A. bornmulleriana*, Bolu-Kökez*)*, B. Trunk of mature tree, C. One-year old female conelet, D. Male cone (*A. nordmanniana,* Şavşat-Artvin) (Forest Tree Seeds and Tree Breeding Research Directorate)

Distribution of Turkish Fir Species

Turkey is uniquely positioned within three major phytogeographical regions—Euro-Siberian, Mediterranean, and Irano-Turanian—and hosts over 10,500 plant taxa, including approximately 3,500 endemics (Kaya & Raynal, 2001). Fir species are well represented, including several endemic taxa with low conservation concern (Ekim et al., 2000).

Key native fir species in Türkiye include:

- **Uludağ Fir (*A. nordmanniana* subsp. *bornmulleriana*)**: Found between 1000–2000 m elevations in the Black Sea and southeastern Marmara regions; shows adaptation to both moist and drier habitats (Saatçioğlu, 1969).
- **Kazdağı Fir (*A. nordmanniana* subsp. *equi-trojani*)**: Endemic to Mount Ida, growing at 400–1650 m; covers roughly 3600 hectares and is vital for breeding efforts due to its adaptability (Kaya et al., 1997).
- **Cilician Fir (*A. cilicica*)**: Native to southern and central Anatolia, includes two subspecies (*subsp. isaurica* in the western Taurus and *subsp. cilicica* in the eastern Taurus), often co-occurring with cedar and juniper (Bozkış, 1988; Doğan et al., 2010).
- **Nordmann Fir (*A. nordmanniana* subsp. *nordmanniana*)**: Grows in the northeast, between 1000–2000 m, commonly associated with beech and spruce. A local variant from the Çataldağ area is notable for its distinctive wood and pollen traits (Ata & Merev, 1987).

Economic and Medicinal Significance

Turkish firs are valuable for their technical wood properties, especially in the furniture and paper sectors, due to their resin-free and light-colored wood (Anşin & Özkan, 1997). Heat-treated Uludağ Fir is especially favored in the timber industry (Yaltırık & Efe, 2000). The resin from *A. cilicica* has been traditionally used in folk medicine for its antiseptic and anti-inflammatory properties, including the treatment of ulcers, asthma, and wounds (Baytop, 1999). *A. nordmanniana* is popular across Northern Europe as a Christmas tree and has also been investigated for its potential as a biosorbent for heavy metal removal from wastewater (Nielsen, 1993; Serencam et al., 2000).

Molecular Markers and cpDNA of Plants

The development of molecular markers, coupled with advancements in statistical modeling and specialized bioinformatics tools, has significantly contributed to the identification of genetic loci associated with complex traits. These innovations have enhanced marker-assisted selection strategies in plant breeding programs (Bernardo, 2008). In forest genetics and tree improvement studies, DNA-based markers have largely replaced traditional morphological and biochemical markers due to their high polymorphism and independence from environmental influences (Neale et al., 1992; Aronen, 1995).

In plant molecular biology, three types of DNA are commonly used for genetic studies: nuclear DNA (nDNA), chloroplast DNA (cpDNA), and mitochondrial DNA (mtDNA) (Simpson, 2006). Among these, cpDNA is particularly favored in phylogenetic and evolutionary studies because of its relatively conserved structure and its higher mutation rates in non-coding regions compared to other genomes (Palmer et al., 1988; Taberlet et al., 1991; Shaw et al., 2007). The chloroplast genome is also easy to isolate and sequence, making it a suitable and practical target for studies on plant evolution and systematics (Liang, 1997).

*mat*K Gene

The *matK* gene (maturase K) has become one of the most widely utilized markers in plant phylogenetics due to its relatively high sequence variability across taxonomic levels ranging from families to individual species. Positioned within the large single-copy (LSC) region of the chloroplast genome, adjacent to the inverted repeat regions, the *matK* gene spans approximately 1,500 base pairs and contains an open reading frame that facilitates its amplification and analysis (Hilu & Barthet, 2008).

Because of its high resolution and variability, especially in non-coding flanking regions, *matK* is frequently applied in phylogenetic and taxonomic studies of angiosperms. In some cases, researchers amplify not only the coding *matK* sequence but also its surrounding intronic regions—particularly the entire *trnK* intron—to obtain longer, more informative sequences ranging from 2,400 to 2,700 bp (Müller & Borsch, 2005a; Wanke et al., 2006). These extended sequences enhance both interspecific and intraspecific analyses.

Consequently, *matK* has proven to be a practical and widely accepted genetic marker for species identification in plants, particularly in DNA barcoding and phylogenetic studies (CBOL Plant Working Group, 2009) (see Figure 2).

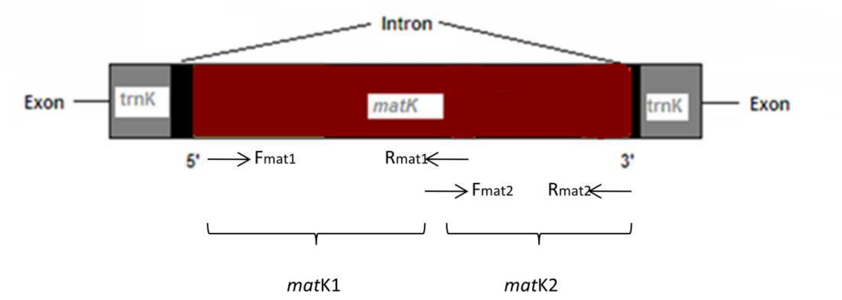

Figure 2 Position of *mat*K gene region on chloroplast DNA (Adapted from Li *et.al*.1997).

Objective of the Study

This study aims to explore the evolutionary patterns and phylogenetic relationships of Turkish fir species through the analysis of the *matK* region within their chloroplast DNA. By examining sequence variation in this genomic region, the research seeks to clarify taxonomic uncertainties and contribute to a better understanding of the genetic structure of these taxa.

Additionally, the study evaluates the diversity patterns of the *matK* region among Turkish firs and compares them with global *Abies* species to determine evolutionary divergence and genetic relatedness between firs from different geographic regions, including Europe, Asia, and North America.

MATERIALS AND METHODS

Plant Material

This research was conducted using open-pollinated seeds collected from six different *Abies* taxa native to various regions across Türkiye (Table 1). The seed material was provided by the Forest Tree Seeds and Tree Breeding Research Directorate, affiliated with the Ministry of Environment and Forestry. The seeds were extracted from mature cones and stored under refrigerated conditions at +4°C until use. For each population, seed samples were obtained from approximately 20 to 25 mature trees to ensure representative genetic diversity.

Detailed information regarding the geographic origin, altitude, and coordinates of the sampled populations was compiled with the support of the aforementioned institution and is summarized in Table 1.

Table 1. The number of Turkish fir populations included this study.

Studied *Abies* Species in Turkey	**Number of Population**
***Abies nordmanniana* subsp. *nordmanniana* (ANN)**	**3**
***Abies nordmanniana* subsp. *bornmuelleriana* (endemic) (ANB)**	**5**
***Abies nordmanniana* subsp. *equi-trojani* (endemic) (ANE)**	**3**
***Abies cilicica* subsp. *isaurica* (endemic) (ACI)**	**3**
***Abies cilicica* subsp. *cilicica* (ACC)**	**3**
***Abies x olcayana* (endemic) (AXO)**	**1**

Extraction of DNA from Seeds

To represent each population, seeds were collected from five different maternal trees, with roughly 20 to 25 seeds taken per tree. Genomic DNA was extracted from the embryos within the seeds using the CTAB (cetyltrimethylammonium bromide) method as outlined by Doyle and Doyle (1990). The extracted DNA was assessed for integrity via 2% agarose gel electrophoresis, and intact samples were stored at –20°C in labeled Eppendorf tubes for later use.

In this study, two primer sets developed by Gülsoy (2010) using Primer3 software were used to amplify the *matK* gene due to their efficiency in PCR amplification. Although alternative primers for *matK* exist in the literature (Suyama et al., 2000; Gadek et al., 1996; Wang et al., 2000; Li et al., 1997), the following sequences were selected for their reliability:

*mat*K1-Forward Primer (Fmat1) :
5‘GATCCTGTATCTTTTGCCAGGA 3‘

Reverse Primer (Rmat1) :
5‘ GAACCTTTCGTCGCTGGAT 3‘

*mat*K 2-Forward Primer (Fmat2) :

5‘ CTTTCGGGACGACAATAATC 3‘
Reverse Primer (Rmat2) :
5‘ CGAGCTTCTGTTCCTCGTT 3‘

PCR Amplification, Gel Electrophoresis, Sequencing and Data Analysis

Polymerase Chain Reaction (PCR) amplifications were carried out in 25 µL reaction volumes, each containing template DNA, magnesium chloride ($MgCl_2$), dNTPs, and the respective primers. Optimization of reaction conditions was performed using an Eppendorf Mastercycler (Eppendorf, Canada).

To assess amplification success, 5 µL of each PCR product was run on a 1% agarose gel stained with ethidium bromide (5 µg/mL) and electrophoresed in 1X TBE buffer at 90 volts for 30 minutes. DNA bands were visualized and photographed using a Vilber Lourmat imaging system (France). Amplified *matK* fragments were stored at –20 °C prior to sequencing.

Sequencing was performed using both forward and reverse primers. Prior to sequencing, PCR products were purified using the Nucleospin Extract Kit (Clontech Laboratories, Inc.). Sequencing reactions were conducted at Refgen Biotechnology (METU Teknokent, Ankara) using the BigDye Cycle Sequencing Kit and ABI 310 Genetic Analyzer (PE Applied Biosystems), following manufacturer protocols.

The *matK* gene was amplified in two segments using the four primer pairs described earlier. Raw chromatograms were evaluated using FinchTV v1.4.0 (Patterson et al., 2004–2006), and sequence alignments were generated through multiple alignment strategies followed by manual or software-guided corrections. Any unresolved ambiguities were either corrected or the problematic samples excluded from the dataset.

Final sequence files were exported in FASTA format. Unreadable bases were marked as “N,” and alignment gaps were represented with dashes “–”. Trimming was performed at both ends to eliminate low-quality reads, resulting in shortened but high-confidence sequences.

Molecular diversity indices and phylogenetic analyses were conducted using MEGA version 5 (Tamura et al., 2011). Additionally, to allow for broader phylogenetic comparison, *matK1* and *matK2* sequences from various *Abies* species were retrieved from the NCBI database (as of 2011). GenBank accession numbers and species names are listed in Table 2.

Table 2 Abies species and their gene bank accession numbers of the matK sequences in NCBI data bank.

Species name	**Gene bank accession numbers**
Abies alba	HQ619823
Abies hidalgensis	EU269026.1
Abies bracteata	AF456365.1
Abies holophylla	AF143441.1
Abies firma	AF143436.1
Abies numidica	AB019864.1
Abies veitchii	AB029669.1
Abies sibirica	AB029668.1
Abies sachalinensis	AB029667.1
Abies nephrolepis	AB029666.1
Abies mariesii	AB029665.1
Abies lasiocarpa	AB029664.1
Abies koreana	AB029663.1
Abies homolepis	AB029662.1
Abies fraseri	AB029660.1
Abies fargesii	AB029658.1
Abies fabri	AB029657.1

Neighbor-Joining Method and Phylogenetic Tree Construction

The Neighbor-Joining (NJ) method is a widely used algorithm in molecular systematics for constructing phylogenetic trees based on distance matrices. Developed by Saitou and Nei (1987), the NJ approach uses a bottom-up clustering strategy to estimate tree topology and branch lengths, starting from a star-like configuration and progressively merging the closest pairs of Operational Taxonomic Units (OTUs).

Unlike traditional minimum-evolution methods, which evaluate all possible tree topologies to find the one with the shortest overall branch length, the NJ method dynamically minimizes branch lengths at each clustering step. Although it may not always produce the absolute minimum-evolution tree, it is recognized for its computational efficiency and high accuracy in many practical cases.

In this study, phylogenetic trees were constructed using MEGA version 5 software. The NJ algorithm was applied to the aligned *matK* sequences to explore the genetic relationships among Turkish *Abies* taxa and to compare them with other species retrieved from GenBank.

RESULTS

PCR amplification of the chloroplast matK regions in Turkish fir samples using the selected primer sets yielded distinct and reproducible bands, indicating successful amplification. Among the two targeted segments, the regions amplified by the matK1 and matK2 primer pairs were chosen for sequencing due to their clarity and quality (Figure 3)

Molecular Diversity among *Abies* Species with Respect to *mat*K Region

While the full matK gene typically ranges between 1500 and 1800 base pairs, this study focused on sequencing partial regions of the gene. It was anticipated that the combined use of matK1 and matK2 primers would capture a continuous fragment. Ultimately, a 1215 bp segment was successfully amplified and sequenced, covering parts of both regions. This total length was slightly shorter than the expected full gene sequence (Figure 3)

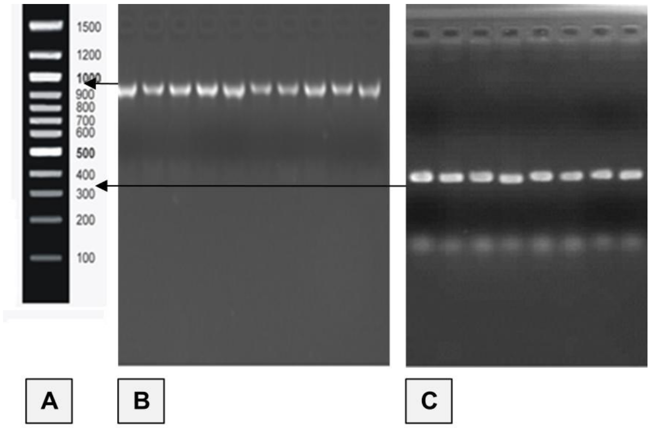

Figure 3 Photograph showing the amplified DNA of two *mat*K regions of cpDNA. A is the DNA ladder, B is *mat*K1 with 913bp and C is *mat*K2 with 302bp.

Sequence Characteristics and Genetic Variation

The combined *matK* region sequenced from Turkish firs measured 1215 base pairs in length, with an overall GC content of 35.2%. Upon analysis, this region exhibited no detectable sequence variation among the sampled taxa. All nucleotide positions were fully conserved, and there were no variable sites, parsimony-informative sites, or singletons identified across the examined sequences.

When analyzed separately, the *matK1* segment was 913 bp long and showed a GC content of 38.7%, while the *matK2* fragment measured 302 bp and had a slightly lower GC content of 36%. Despite targeting different regions of the gene, both segments displayed high sequence conservation within Turkish fir populations.

Comparative Analysis of Global *Abies* Species Using matK1 Region

Although the genus *Abies* includes over 50 recognized species worldwide, complete *matK1* sequences are available for only 17 of these in public databases. To assess the evolutionary placement of Turkish firs within the broader context of global *Abies* diversity, *matK1* sequences from these species were obtained from the NCBI GenBank repository.

Due to differences in the availability and completeness of sequences across species, only overlapping regions of the *matK1* gene—measuring 615 base pairs—were used for comparative analysis. This standardized segment exhibited a GC content of 34.5% across the global species dataset.

Alignment and sequence variation analysis revealed that out of the 615 sites, 607 were conserved, while 8 sites showed variability. These included 3 singleton positions and 5 parsimony-informative sites. The transition/transversion bias (R) was calculated as 3.279, indicating a notable preference for transitions over transversions.

In contrast to other species, Turkish firs were found to form a genetically distinct cluster based on this segment, particularly when compared to Asian and North American firs. Complete *matK* sequences were available for only three Asian species—*A. veitchii*, *A. firma*, and *A. holophylla*. These species were used for additional comparisons based on the *matK2* region.

Global Comparison Based on the matK2 Region

The comparative evaluation of the *matK2* region included three Asian fir species—*Abies veitchii*, *A. firma*, and *A. holophylla*—for which full *matK* sequences were publicly available through NCBI. A 252 base pair overlapping segment was extracted for alignment with Turkish fir sequences.

Analysis of this fragment revealed a highly conserved structure across species, with only two variable sites detected. Among these, one was a singleton site and the other was parsimony-informative. These findings further supported the genetic distinctiveness of Turkish firs, as they clustered separately from the Asian species in this region as well.

Overall, both matK1 and matK2 comparisons indicated low sequence variability across *Abies* taxa globally, yet Turkish firs consistently grouped as a cohesive, monophyletic unit distinct from other geographic lineages.

Phylogenetic Tree Construction of Turkish Fir Species

Genetic distances among all Turkish *Abies* samples were calculated using the MEGA 5.0 software. Since the *matK* sequences from different populations showed no detectable variation, a single representative sequence was selected from each taxon for the purpose of phylogenetic tree construction(Figure 4)

The resulting tree, developed using the Neighbor-Joining (NJ) method, incorporated bootstrap support values and corresponding branch lengths to ensure robustness of the inferred relationships. To root the tree and provide an evolutionary outgroup, *Tsuga diversifolia* (GenBank accession number EF395589.1) from the same family (Pinaceae) was included.

As expected, all Turkish fir taxa formed a single, well-supported monophyletic cluster based on the partial *matK* sequences, indicating strong genetic similarity and a shared chloroplast lineage among them.

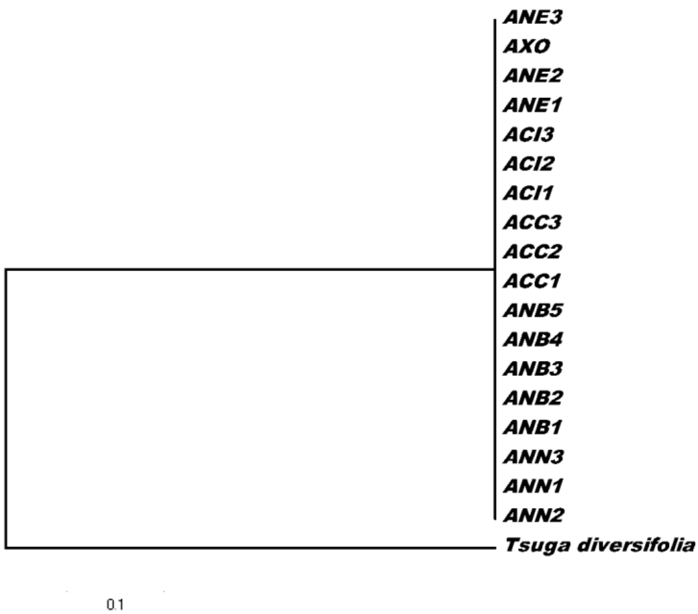

Figure 4 Phylogenetic tree was constructed by using neighbor joining method for *Abies* spp. in Turkey

Global Phylogenetic Tree of *Abies* Species Based on matK1 Region

A Neighbor-Joining tree was constructed using the aligned matK1 sequences of 17 *Abies* species obtained from GenBank. This tree revealed three main clades within the genus, each reflecting distinct geographic affiliations (Figure 5).

Turkish firs formed a clearly defined monophyletic group that clustered closely with *Abies* numidica, a Mediterranean species found in North Africa. This clade represented the European-Mediterranean lineage. A second major grouping consisted primarily of Asian species, while a third clade included a mixture of Asian and North American species such as A. lasiocarpa and A. fraseri. Notably, A. mariesii from Japan was positioned near a smaller subclade comprising A. hidalgensis, A. bracteata (North America), and A. alba (Europe), suggesting some degree of shared ancestry across continents.

These patterns reinforce the genetic distinctiveness of Turkish firs and their closer relationship to other western Eurasian taxa than to their Asian or American counterparts.

Global Phylogeny Based on matK2 Region and Comparison with Morphological Classification

A second phylogenetic tree was constructed using the *matK2* sequences from Turkish firs alongside those of *Abies veitchii*, *A. firma*, and *A. holophylla*—the only species with complete *matK* data available from Asia. This analysis further emphasized the genetic uniqueness of Turkish firs, which clustered separately from their East Asian counterparts (Figure 6).

Interestingly, the genetic groupings inferred from chloroplast *matK* sequences did not align perfectly with classical morphological classifications proposed by Farjon (1990) and Liu (1971). While traditional systems often relied heavily on geographic distribution and phenotypic traits, the molecular evidence presented here suggests that biogeographical proximity does not necessarily dictate evolutionary relatedness in *Abies*.

Instead, Turkish firs appeared more closely related to other European and Mediterranean taxa, supporting the idea of a distinct western Eurasian lineage that diverged from Asian and North American clades.

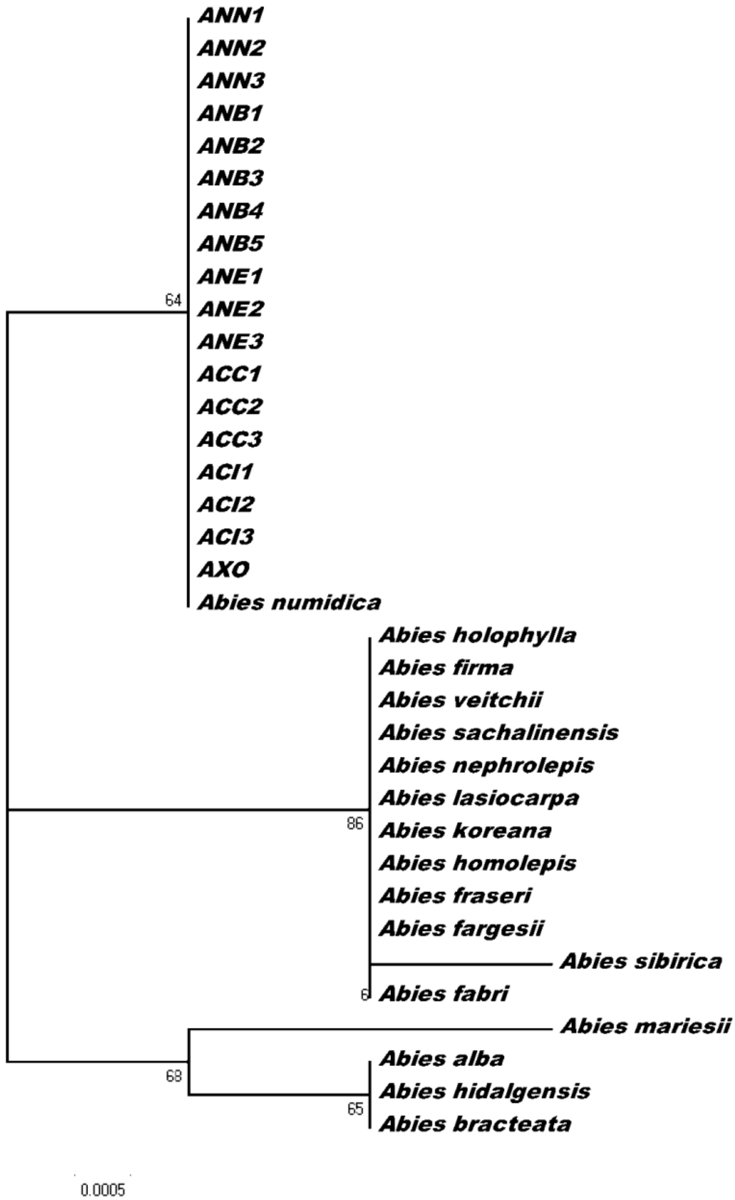

Figure 5 Phylogenetic tree was constructed by using neighbour joining method for *Abies* spp. based on *mat*K1 region.

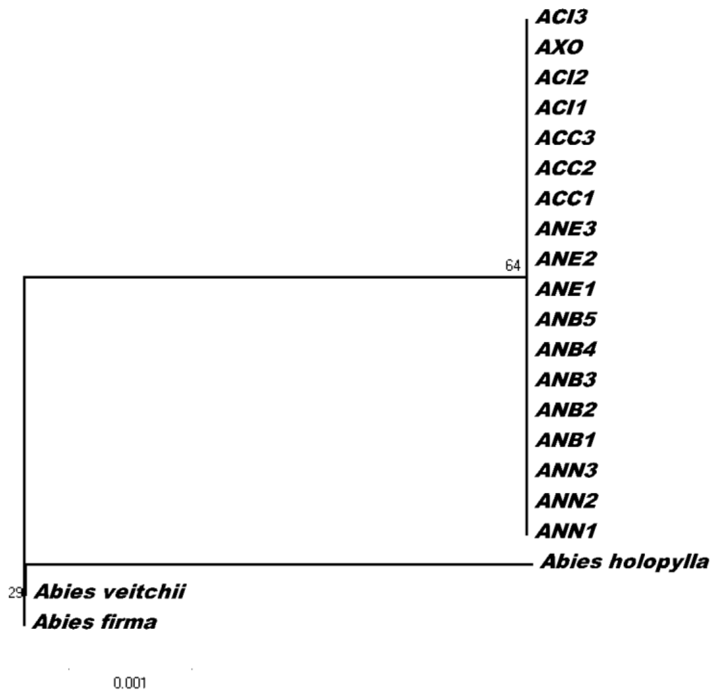

Figure 6 Phylogenetic tree was constructed by using neighbour joining method for *Abies* spp. based on *matK2* region

DISCUSSION

Molecular Diversity Observed in the matK Region

In this study, a 1215 bp fragment of the *matK* gene was successfully amplified and sequenced from Turkish fir species. Although this length is shorter than the 1551 bp reported for Pinaceae by Wang et al. (2000), it exceeds the 615 bp region analyzed by Suyama et al. (2000). Such variation in reported lengths across studies can often be attributed to insertions, deletions, or differences in primer design.

Despite the adequate sequence length, no polymorphism was detected among Turkish fir taxa. The *matK* region appeared completely conserved, showing no variable or parsimony-informative sites. This result indicates a high degree of sequence stability within the chloroplast genomes of these species.

Nevertheless, phylogenetic analysis using the *matK1* region successfully grouped Turkish firs with European species, suggesting a closer evolutionary link to western Eurasian lineages. The *matK2* data also supported the separation of Turkish firs from East Asian firs, further reinforcing their genetic distinctiveness.

Phylogeny of the Genus *Abies*

Numerous classification systems have been proposed for the genus *Abies* over the years. The most widely accepted frameworks include those by Liu (1971), and subsequent revisions by Farjon and Rushforth (1989), followed by Farjon (1990). However, due to the morphological complexity and overlapping traits within the genus, taxonomic boundaries remain inconsistent across these systems.

In this study, the chloroplast *matK* region was employed to clarify the evolutionary relationships among six Turkish *Abies* taxa: *A. nordmanniana* subsp. *nordmanniana*, subsp. *bornmuelleriana*, subsp. *equi-trojani*, *A. cilicica* subsp. *isaurica*, subsp. *cilicica*, and the hybrid *A.* × *olcayana*. Phylogenetic reconstruction using the Neighbor-Joining method revealed that all Turkish firs formed a monophyletic clade, showing no sequence-level divergence among them.

This clustering pattern supports the presence of a shared ancestral chloroplast lineage and reflects the high level of genetic conservation in the *matK* region within Turkish fir populations. These results are in agreement with earlier findings by Fady and Conkle (1993), who also reported limited genetic variation among *A. nordmanniana* populations.

Phylogeny of Turkish *Abies* and Closely Related Species

To better understand the evolutionary placement of Turkish fir species within the global *Abies* lineage, publicly available *matK* sequences from multiple fir taxa were retrieved from the NCBI GenBank database (as of 2011). Comparative phylogenetic analysis revealed that Turkish firs consistently clustered together as a distinct monophyletic group and showed the closest affinity to the North African species *Abies numidica*, indicating a Mediterranean-European lineage.

These findings are consistent with earlier studies, such as Fady and Conkle (1993), who reported a strong genetic relationship between *A. alba* and

A. nordmanniana subsp. *bornmuelleriana*. Similarly, Scaltsoyiannes et al. (1999) demonstrated comparable clustering patterns through allozyme analyses.

While previous studies utilizing various molecular markers—such as nuclear SSRs, mitochondrial DNA, or alternative chloroplast regions like *rbcL*—have sometimes shown different patterns of differentiation (e.g., Suyama et al., 2000; Ziegenhagen et al., 2005; Parducci & Szmidt, 1999), the low sequence variability observed in the *matK* region in this study limited species-level resolution.

In contrast, more polymorphic markers used in other studies (e.g., cpSSRs, mtDNA introns, or tandem repeats) revealed clearer genetic structure and differentiation among Mediterranean fir species (Kaya et al., 2008; Hansen et al., 2005; Liepelt et al., 2010). These discrepancies highlight the importance of marker choice and sequence length in phylogenetic inference. The highly conserved nature of the *matK* gene in Turkish firs may explain why the results here diverge from some previous classifications.

CONCLUSION

The primary aim of this study was to generate genetic data from the chloroplast *matK* region to clarify the taxonomic status and evolutionary relationships of Turkish fir species. A partial *matK* sequence approximately 1215 base pairs in length was obtained, yet no sequence variation was detected among the six examined taxa. This indicates that the *matK* region is highly conserved within Turkish firs and does not provide sufficient resolution for distinguishing species at the intraspecific level.

Despite this limitation, the analysis of *matK* sequences from global *Abies* species offered valuable phylogenetic insights. The results supported traditional classifications by Liu (1971) and Farjon (1990), placing Turkish firs within the section *Abies* and revealing a closer genetic affinity to European and Mediterranean species than to their Asian or North American counterparts.

In summary, although the *matK* gene region alone does not offer adequate variability for resolving fine-scale taxonomic distinctions among Turkish firs, it remains a useful marker for broader phylogenetic assessments across the genus. To achieve greater taxonomic resolution within this complex group, future research should focus on more variable chloroplast regions or incorporate nuclear genetic markers.

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CHAPTER 16

POSTHARVEST AFLATOXIN DEVELOPMENT IN TREE NUTS: RISK FACTORS, PREVENTION STRATEGIES, AND HEALTH IMPLICATIONS

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1. INTRODUCTION

Tree nuts are agricultural products of strategic global importance due to their rich nutritional content and economic value. Species such as almond (Prunus dulcis), walnut (Juglans regia), hazelnut (Corylus avellana), pistachio (Pistacia vera), and chestnut (Castanea sativa) are critical in human nutrition owing to their high contents of healthy fats, proteins, dietary fibers, vitamins (especially vitamin E and folate), and minerals (such as magnesium and potassium) (Ros, 2010; Bolling et al., 2011). Owing to its favorable climate and geography, Türkiye is among the global leaders in the production of hazelnuts and pistachios, holding a significant share in the international market (FAO, 2022).

One of the most serious problems encountered throughout the production-to-consumption chain of tree nuts is postharvest mycotoxin contamination. Among these, aflatoxins are considered the most critical toxic compounds threatening both product quality and food safety (Kabak et al., 2006). Aflatoxins are chemically polyketone mycotoxins synthesized by certain molds, primarily Aspergillus flavus and Aspergillus parasiticus, under favorable temperature and humidity conditions (Payne & Brown, 1998). Aflatoxin B_1 (AFB_1) is the most toxic among them, capable of causing severe health effects including hepatotoxicity, mutagenicity, and carcinogenicity (Wild & Gong, 2010). The International Agency for Research on Cancer (IARC), under the World Health Organization (WHO), has classified AFB_1 as a Group 1 human carcinogen (IARC, 2012).

Aflatoxin contamination not only poses a threat to public health but also leads to significant economic losses and export restrictions due to exceedance of permissible limits in international trade (Pitt & Miller, 2017). Therefore, controlling aflatoxin formation in tree nuts is of paramount importance for both food safety and sustainable agricultural production.

2. CHEMICAL STRUCTURE, BIOSYNTHESIS, AND TOXICITY OF AFLATOXINS

Aflatoxins are secondary metabolites within the broader category of mycotoxins and are noted for their toxic, mutagenic, teratogenic, and especially carcinogenic properties. The health risks associated with these compounds have been well documented by the WHO and IARC, with Aflatoxin B_1 (AFB_1)

specifically classified as a Group 1 carcinogen (IARC, 2012). Aflatoxins are mainly produced by soil-borne filamentous fungi such as Aspergillus flavus and Aspergillus parasiticus (Frisvad et al., 2007; Klich, 2007). Under uncontrolled environmental conditions involving temperature, humidity, and oxygen, these toxins can develop in nuts such as hazelnuts, almonds, pistachios, and walnuts, thereby posing serious threats to both human health and livestock production systems (Wild & Gong, 2010; Wu et al., 2014).

Chemically, aflatoxins possess polyketide structures and are complex organic molecules containing difuran rings. The most commonly studied types—AFB_1, B_2, G_1, and G_2—are named for their fluorescent properties under ultraviolet (UV) light. Among them, AFB_1 is the most toxic and prevalent, shown in numerous studies to cause liver damage, immunosuppression, and hepatocellular carcinoma (Wild & Gong, 2010; IARC, 2012).

The biosynthesis of aflatoxins involves a complex sequence of genetic and enzymatic reactions. The aflatoxin biosynthesis gene cluster comprises approximately 25 genes, among which aflR, a transcriptional activator, and aflS, a co-regulator, play critical roles in initiating the process (Yu et al., 2004; Amaike & Keller, 2011). The pathway begins with the conversion of acetyl-CoA into norsolorinic acid via polyketide synthase, followed by a series of reduction and cyclization steps leading to the formation of AFB_1 and related compounds (Cary et al., 1996). This biosynthetic pathway is highly sensitive to environmental factors such as temperature (25–35°C), relative humidity (>70%), substrate composition, and oxygen availability (Medina et al., 2017).

The toxicological impacts of aflatoxins on human and animal health are extensive. The liver is the primary target organ, where AFB_1 is metabolized by cytochrome P450 enzymes into the highly reactive AFB_1-8,9-epoxide. This metabolite forms covalent bonds with DNA, inducing mutations—particularly in the TP53 gene—which play a key role in the development of hepatocellular carcinoma (Groopman et al., 2008; Kew, 2013). Aflatoxin toxicity can manifest in both acute and chronic forms. Acute toxicity, resulting from high-dose exposure, can cause liver necrosis, hemorrhage, edema, and even death (Liu & Wu, 2010). Chronic exposure, on the other hand, is associated with hepatocarcinogenesis, growth retardation, immunosuppression, and neurological disorders (Williams et al., 2004).

To mitigate the health risks posed by aflatoxins, international food safety authorities have established maximum allowable limits in food products. The European Union, for example, limits the total aflatoxins ($AFB_1 + B_2 + G_1 + G_2$) in tree nuts to a maximum of 10 µg/kg and AFB_1 alone to 8 µg/kg (EC, 2006). Similar regulations are enforced under the Turkish Food Codex (TGK, 2022) to ensure consumer protection.

Postharvest aflatoxin development is a major concern for tree nut quality. Improper drying, storage, and transportation conditions provide a conducive environment for the proliferation of Aspergillus species and aflatoxin production (Pitt & Hocking, 2009). Environmental parameters such as moisture content, temperature, mechanical integrity of the nut, storage duration, and oxygen availability are key determinants of aflatoxin formation (Battilani et al., 2016). Furthermore, climate change-driven shifts in temperature and humidity patterns have exacerbated the prevalence of Aspergillus spp., thereby increasing aflatoxin risks (Battilani et al., 2016; Medina et al., 2017).

The literature emphasizes that the postharvest phase is critical in preventing aflatoxin contamination in tree nuts. Effective and rapid drying, controlled storage temperature and humidity, prevention of mechanical damage, and proper packaging are among the primary preventive strategies (Magan et al., 2003). Moreover, the use of biological control agents and chemical inhibitors to suppress Aspergillus growth has gained attention in recent years (Yu et al., 2005).

In this context, a thorough understanding of the biochemical mechanisms underlying postharvest aflatoxin development, the identification of risk factors, and the implementation of effective control strategies are essential for ensuring food safety. This review aims to synthesize recent findings in the field and serve as a comprehensive guide for producers, food safety professionals, and policymakers.

3. POST-HARVEST FACTORS INFLUENCING AFLATOXIN DEVELOPMENT IN TREE NUTS

Tree nuts—particularly almonds (Prunus dulcis), hazelnuts (Corylus avellana), walnuts (Juglans regia), and pistachios (Pistacia vera)—are of significant nutritional and economic value due to their high content of fats, proteins, and bioactive compounds (Özdemir & Devres, 1999). However, this rich composition also provides a conducive environment for the growth of mycotoxigenic fungi, especially those producing aflatoxins. Species such as Aspergillus flavus and Aspergillus parasiticus can colonize these products under favorable environmental conditions and synthesize toxic metabolites (Pitt & Hocking, 2009).

Post-harvest processes play a critical role in determining the extent of aflatoxin contamination. These processes include harvest timing, drying methods, storage conditions, and practices throughout the transportation and processing chain. Improper practices at any of these stages can increase the risk of aflatoxin formation, posing a threat to both product quality and public health (Magan et al., 2003; Hell et al., 2000). Therefore, effective aflatoxin risk management requires a comprehensive evaluation of post-harvest factors.

3.1. The Role of Harvest Timing in Aflatoxin Formation

Harvest timing is one of the most critical factors in preventing aflatoxin contamination. When tree nuts reach biological maturity, their lipid content peaks, providing an ideal substrate for mycotoxigenic fungi (Horn, 2003). If nuts are not harvested promptly, their prolonged exposure to ambient Aspergillus spores increases the likelihood of contamination.

Conversely, nuts harvested prematurely may not be physiologically mature, often containing high moisture levels that hinder adequate drying. Incomplete drying can promote microbial activity and consequently stimulate aflatoxin biosynthesis (Kumar et al., 2008). Therefore, determining the optimal harvest time by considering both physiological and environmental conditions is a fundamental step in minimizing aflatoxin risk (Bhat et al., 2010).

Moreover, the optimal harvest time varies among nut species depending on phenological development, local climate conditions, and agricultural practices. Thus, species- and region-specific integrative harvest scheduling strategies are crucial tools in aflatoxin management (Medina et al., 2015).

3.2. Effects of Drying Methods on Aflatoxin Development

Drying is one of the most essential and effective methods for preventing aflatoxin biosynthesis in tree nuts. Although traditional sun drying is commonly used due to its low cost and accessibility, its effectiveness is highly dependent on environmental factors. Nuts dried on moist surfaces or under insufficient sunlight facilitate the growth of Aspergillus species, thereby increasing aflatoxin production (Kabak et al., 2006; Medina et al., 2014). Uneven drying during traditional methods may also leave residual moisture, creating favorable conditions for microbial proliferation (Magan & Aldred, 2007).

Modern drying technologies enable rapid reduction of moisture content below 7% through controlled temperature and humidity parameters, thereby inhibiting Aspergillus growth and aflatoxin biosynthesis (FAO, 2018; Magan & Aldred, 2007). Techniques such as hot air drying, vacuum drying, and microwave-assisted drying not only reduce aflatoxin risks but also help preserve the nutritional and organoleptic qualities of the nuts (Magan et al., 2003). Their ability to shorten drying time and improve energy efficiency makes them favorable for commercial use (FAO, 2018).

3.3. Impact of Storage Conditions on Aflatoxin Formation

Storage is a critical phase where aflatoxin contamination can persist or even escalate. The optimal temperature range for the activation of Aspergillus spores and aflatoxin production is typically between 25–35 °C, with relative humidity levels above 70% favoring fungal growth (Magan et al., 2003; Hell et al., 2000). Under such conditions, spore germination accelerates, enhancing mycotoxin biosynthesis.

Inadequate ventilation, localized moisture accumulation, and temperature fluctuations further promote fungal development (Bankole & Adebanjo, 2004). Therefore, modern storage practices emphasize the importance of temperature and humidity control. Cold storage (4–10 °C), vacuum packaging, and oxygen limitation are effective strategies that significantly restrict the metabolic activities of Aspergillus species and thereby limit aflatoxin formation (Ayranci & Karaca, 2021).

Regular quality control and mycological analyses during storage also enable early detection of aflatoxin risk, allowing for timely preventive measures (Ezekiel et al., 2018).

3.4. Role of Mechanical Damage and Biological Factors

Aflatoxin contamination in tree nuts is significantly influenced not only by environmental conditions but also by physical damage and biological pests. Mechanical injuries such as shell cracks, bruises, and insect holes during harvesting, transportation, and processing compromise the natural protective barrier of the nuts. This facilitates fungal penetration, particularly by

Aspergillus flavus, and increases susceptibility to aflatoxin contamination (Sweeney & Dobson, 1998). Such damage promotes fungal colonization and enhances toxin production (Horn, 2003; Medina et al., 2014).

In addition to mechanical damage, biological pests play a critical role in aflatoxin development. Insects such as the navel orangeworm (Amyelois transitella), hazelnut weevil (Curculio spp.), and walnut husk fly inflict physical injuries while also acting as vectors for the dissemination of Aspergillus spores. Secondary infections induced by these pests significantly elevate aflatoxin contamination risk (Safe Food Alliance, 2020).

Studies on high-value products like almonds and pistachios have shown a positive correlation between insect damage and aflatoxin levels. The Safe Food Alliance (2020) reported that aflatoxin concentrations were significantly higher in insect-damaged nuts compared to healthy ones. Therefore, integrated pest management strategies combined with careful handling during harvesting and transportation are vital measures in aflatoxin control.

3.5. Aflatoxin Risk Management During Transportation and Processing

The post-harvest chain, from the field to the consumer, involves transportation and processing steps that require meticulous aflatoxin risk management. Exposure to humid environments or prolonged retention in warm, enclosed spaces promotes Aspergillus development and aflatoxin formation (Hell et al., 2000). Lack of adherence to hygiene standards during transportation further exacerbates microbial contamination on nut surfaces (Tirado et al., 2010).

Strict hygiene compliance in processing facilities is essential, particularly during shelling, roasting, and packaging, where appropriate control of temperature and humidity is critical to preventing aflatoxin formation. Aspergillus flavus and Aspergillus parasiticus proliferate rapidly under high humidity and favorable temperatures. Therefore, maintaining moisture levels below 7% and continuously monitoring environmental parameters are crucial for inhibiting fungal activity and aflatoxin biosynthesis. Roasting plays a pivotal role in killing fungal spores and reducing aflatoxin levels; however, inadequate roasting fails to achieve the desired protective effect, thus elevating the risk (Magan & Aldred, 2007).

Additionally, poor hygiene may lead to microbial biofilm formation on raw nut products, facilitating fungal adherence and promoting aflatoxin synthesis. Failure to maintain optimal temperature and humidity during post-harvest storage and processing increases Aspergillus metabolic activity and accelerates toxin production. Therefore, an integrated, interdisciplinary management approach involving rigorous hygiene and environmental control across all processing stages is essential. Such practices help minimize aflatoxin contamination, prevent economic losses for producers, and mitigate health risks for consumers (Magan & Aldred, 2007).

4. DETECTION AND ANALYTICAL METHODS FOR AFLATOXINS IN TREE NUTS

Aflatoxins are metabolites produced by Aspergillus species and are potent mycotoxins capable of causing both acute toxicity and long-term carcinogenesis in humans and animals. The International Agency for Research on Cancer (IARC) has classified aflatoxin B_1 as a Group 1 carcinogen, indicating its confirmed carcinogenicity to humans (IARC, 2012). Tree nuts, particularly almonds, hazelnuts, walnuts, and pistachios, are at high risk of aflatoxin contamination, especially under improper postharvest handling and storage conditions. Therefore, the early detection of aflatoxins is critical for ensuring food safety and meeting commercial standards. This section discusses the major analytical techniques, sampling strategies, and current methodologies used in aflatoxin detection in detail.

4.1. Sampling and Sample Preparation

Due to the heterogeneous distribution of aflatoxins within bulk commodities, sampling procedures directly influence the accuracy of analytical results. Consequently, sampling must be conducted in strict accordance with international standards. Regulations such as Codex Alimentarius (CAC/RM 40-1993) and the European Commission Regulation No. 401/2006 specify minimum sample quantities and homogenization requirements based on batch size. Typically, multiple incremental samples are collected from 20–30 kg lots and homogenized in the laboratory to prepare a representative test portion. This approach reduces sample-based variability and enhances the reliability of results (Codex Alimentarius, 2019).

Given the chemical stability of aflatoxins, extraction is usually performed using organic solvents such as acetonitrile-water mixtures. The crude extract is then purified using immunoaffinity columns (IAC) or solid-phase extraction (SPE) techniques to improve selectivity and remove matrix interferences. These purification steps are essential for enhancing the sensitivity and accuracy of instrumental analyses (AOAC, 2016).

Effective sample preparation is thus crucial to obtaining valid and reliable analytical data for both producers and consumers.

4.2. Qualitative and Semi-Quantitative Detection Methods

Qualitative and semi-quantitative analytical methods for aflatoxin detection play a vital role in preliminary screening and field applications. These techniques are valued for their rapidity and cost-effectiveness, serving as an initial evaluation tool prior to confirmatory laboratory analyses.

Thin-layer chromatography (TLC) is a classic method used for the qualitative or semi-quantitative determination of aflatoxins. In this technique, aflatoxins extracted with organic solvents are applied to silica gel-coated glass plates and separated using appropriate solvent systems. Aflatoxins can then be visualized under UV light at 365 nm as blue-green fluorescent spots. While TLC offers the advantages of simplicity and low instrumentation requirements, its limited sensitivity and lack of quantitative precision necessitate confirmation with more advanced techniques (AOAC, 2016; Stroka et al., 2000).

Enzyme-linked immunosorbent assay (ELISA) is based on antigen-antibody interactions and allows for the rapid screening of a large number of samples. Although ELISA can yield quantitative data with relatively low detection limits, matrix effects and potential cross-reactivity with other compounds may compromise result accuracy. Therefore, ELISA results are recommended to be confirmed using more robust methods such as high-performance liquid chromatography (HPLC) or liquid chromatography-tandem mass spectrometry (LC-MS/MS) (Turner et al., 2009; Shephard, 2008).

Each of these methods serves a complementary function in the analytical process—such as initial screening, risk pre-assessment, or off-site testing. To ensure food safety, it is critical to implement these techniques in the correct sequence with appropriate validation steps.

4.3. Highly Sensitive Quantitative Analytical Methods

High-performance liquid chromatography with fluorescence detection (HPLC-FLD) is among the most widely used methods for the quantitative determination of aflatoxins. As aflatoxins do not exhibit natural fluorescence, derivatization (e.g., with bromine) is required prior to detection (Shephard, 2008). HPLC-FLD offers high specificity and accuracy, with detection limits ranging between 0.1 and 0.5 µg/kg, making it a standard protocol in aflatoxin analysis.

Currently, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) is considered the gold standard in mycotoxin analysis. This method allows for the simultaneous and highly sensitive detection of multiple mycotoxins and is particularly effective in minimizing matrix effects, making it well-suited for tree nut analysis. LC-MS/MS is indispensable in the industry due to its ability to detect aflatoxins at levels as low as 0.1 µg/kg (Sulyok et al., 2006).

Gas chromatography-mass spectrometry (GC-MS) can also be employed for the analysis of volatile derivatives of aflatoxins, although it is less commonly used in routine testing due to complex sample preparation procedures. Nevertheless, GC-MS remains valuable in confirmatory and advanced research settings (Chu, 1996).

4.4. Innovative Analytical Methods and Emerging Technologies

Recent advancements in aflatoxin detection have extended beyond traditional laboratory-based methods, offering faster, more portable, and user-friendly solutions. Among these, nanotechnology-based sensor systems, microfluidic platforms, and electrochemical biosensors have gained significant attention. These technologies integrate nanomaterials with high surface area (e.g., gold nanoparticles, carbon nanotubes, graphene oxide) with biorecognition elements (e.g., antibodies, aptamers, DNA probes), enabling ultra-sensitive detection of toxic compounds such as aflatoxins (Piermarini et al., 2007).

Lateral flow immunoassay (LFIA)-based strip tests are widely used in the field due to their low cost, portability, and ease of use. These devices are especially beneficial in developing countries or agricultural settings, facilitating on-site decision-making and pre-market control. Typically designed for common toxins such as aflatoxin B_1, LFIA tests can provide visual or instrument-assisted results within 5–10 minutes (Kolosova et al., 2006).

Moreover, microfluidic systems (lab-on-a-chip technologies) allow for the simultaneous analysis of multiple analytes using minimal sample and reagent volumes. These systems integrate sample preparation, extraction, purification, and detection into a single platform, enabling rapid on-site analysis of aflatoxins and other mycotoxins (Dungchai et al., 2009).

When applied in field settings for preliminary screening, such innovative technologies can significantly contribute to early intervention and effective control of aflatoxin risks. In the future, it is anticipated that improvements in sensor sensitivity and multiplex detection capabilities will further enhance their adoption and application in food safety monitoring.

5. PREVENTIVE AND MITIGATIVE STRATEGIES FOR AFLATOXIN CONTROL

The prevention of aflatoxin contamination in tree nuts requires an integrated management strategy that spans the entire production chain—from field-level practices to post-harvest handling, storage, and marketing. These strategies are generally categorized into two groups: (1) preventive measures implemented throughout the production process, and (2) post-harvest interventions aimed at reducing toxin levels. Both conventional and modern biotechnological and physicochemical methods are considered within this framework.

5.1. Field-Level Control

The proliferation and activity of Aspergillus flavus and Aspergillus parasiticus, the primary fungi responsible for aflatoxin biosynthesis, are highly influenced by environmental conditions. Stress factors such as drought, high temperatures, and fluctuations in soil moisture can trigger fungal sporulation and aflatoxin production (Cotty & Jaime-Garcia, 2007). Therefore, the implementation of Good Agricultural Practices (GAP), optimization of irrigation based on climatic conditions, maintenance of soil health, and regular monitoring of pests and pathogens are essential to mitigate the risk of fungal infection. These measures reduce environmental stressors that favor mycotoxin formation by creating conditions unfavorable for fungal growth.

5.2. Genetic Resistance

Genetic resistance in tree nut cultivars represents a vital component of aflatoxin management. Studies on the susceptibility of various local and commercial genotypes to aflatoxin contamination have highlighted the importance of selecting resistant or less susceptible varieties for sustainable production (Abbas et al., 2005). Breeding programs that utilize genetic selection and molecular markers provide long-term, strategic solutions for developing aflatoxin-resistant cultivars.

5.3. Biological Control

Biological control strategies have made significant progress in recent years. The application of atoxigenic A. flavus strains in field conditions effectively suppresses toxigenic strains through competitive exclusion, significantly reducing aflatoxin contamination. Successful examples include biocontrol products such as “Afla-Guard” and “AflaSafe,” which have been effectively employed in the United States (Dorner, 2008). These methods offer an environmentally friendly alternative to chemical pesticides and support the development of sustainable aflatoxin control systems.

5.4. Harvest and Post-Harvest Handling

Delayed harvesting can result in cracking of the nut shell, increasing susceptibility to fungal infection and aflatoxin contamination. Therefore, nuts should be harvested at full maturity and without delay. Efficient and rapid post-harvest drying is essential. During sun drying, the moisture content should be reduced to approximately 6–7% to inhibit aflatoxin production (FAO, 2018). Contact with soil should be prevented by using fabric sheets, plastic covers, or elevated platforms. Appropriate protection from nighttime dew and regular stirring are required to ensure uniform drying and reduce moisture accumulation.

5.5. Storage Conditions

Suboptimal storage conditions significantly promote aflatoxin production. The growth of Aspergillus species and aflatoxin biosynthesis are accelerated under high humidity and temperature. Thus, storage areas should maintain relative humidity below 65% and temperatures not exceeding 25 °C (Bankole & Adebanjo, 2004). Products should be stored in breathable materials, with sufficient ventilation and stringent hygiene practices. The use of pest and mold monitoring systems also allows for early detection and timely intervention. During transportation, maintaining airflow and protecting the product from rain and humidity are also essential for minimizing aflatoxin risk.

5.6. Physical and Chemical Interventions

Post-harvest aflatoxin contamination can be reduced through various physical and chemical techniques. Physical sorting methods rely on observable characteristics such as discoloration, density differences, deformation, or shell damage to detect aflatoxin-contaminated nuts. Optical sorting machines are particularly effective for nuts like hazelnuts and walnuts (Marín et al., 2013). Additionally, the brine flotation method is widely used to separate contaminated nuts based on density differences (Battilani et al., 2016).

Among chemical detoxification methods, ammoniation is notable for altering the chemical structure of aflatoxins and reducing their toxicity, especially in animal feed. However, its use in food intended for human consumption is strictly regulated in many countries (FAO, 2018). Furthermore, natural compounds such as citric acid, ascorbic acid, and other organic acids have shown potential in reducing aflatoxin toxicity and inhibiting fungal growth (Murthy et al., 2014). While the practical application of these compounds is still limited, they offer promising alternatives for enhancing food safety and environmental sustainability.

5.7. Innovative Technologies

Ozone treatment has recently emerged as an effective method for the detoxification of aflatoxins in the food industry. Ozone's strong oxidative capacity disrupts the double bonds in aflatoxin molecules, thereby reducing their toxicity. Due to its non-residual nature and rapid decomposition after application, ozone is considered a safe post-harvest detoxification tool (Kim, Yousef & Dave, 1999). Similarly, cold plasma technology has gained attention as an energy-efficient and eco-friendly method for surface decontamination of food products. These technologies exhibit promising potential in reducing microbial load and inactivating toxins. However, further research is needed to assess their practical applicability and impact on food quality. Their compatibility with natural and organic production systems also indicates promising prospects for future widespread adoption.

6. LEGAL REGULATIONS AND INTERNATIONAL FOOD STANDARDS

Aflatoxin contamination in tree nuts poses not only a serious threat to public health but also leads to significant economic losses in international trade. Consequently, numerous countries and international organizations have developed comprehensive legal frameworks to regulate aflatoxin limits. These regulations are critical for consumer protection, public health assurance, and the global marketability of food commodities (FAO/WHO, 2018).

6.1. Toxicological Basis and Regulatory Limits

Among aflatoxins, Aflatoxin B_1 is recognized as a potent hepatocarcinogen and has been classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC, 2012). The severe implications of aflatoxins on human health form the foundation of food safety legislation. When setting legal limits, scientific criteria such as toxicological evaluations, tolerable daily intake (TDI) values, and consumption patterns are taken into consideration (WHO, 2001).

6.2. European Union Regulations

The European Union (EU) enforces one of the strictest regulatory frameworks for mycotoxins. According to Regulation (EC) No 1881/2006, the maximum limit for total aflatoxins ($B_1 + B_2 + G_1 + G_2$) in tree nuts is set at 4.0 μg/kg, while the limit for Aflatoxin B_1 alone is 2.0 μg/kg (European Commission, 2006). For high-risk imports, batch-based analysis is mandated to mitigate aflatoxin contamination. Furthermore, Decision 2010/223/EC requires increased aflatoxin testing for nuts imported from countries such as Iran, Turkey, India, and China. Products like hazelnuts and pistachios exported from Turkey are systematically analyzed, and non-compliant batches are rejected at EU borders (RASFF, 2022).

6.3. Regulations in the United States

In the United States, the Food and Drug Administration (FDA) has established a maximum allowable limit of 20 μg/kg for total aflatoxins in tree nuts (FDA, 2000). Although this is more lenient than EU limits, stricter thresholds apply for animal feed. FDA regulations are based on toxicological risk assessments and are typically expressed as "action levels." The United

States also permits the use of certain detoxification methods, such as ammoniation, under specific conditions, although their application in food intended for human consumption is highly restricted. Meanwhile, the U.S. Department of Agriculture (USDA) promotes the use of biological control agents to reduce aflatoxin risks (USDA, 2021).

6.4. National Standards in Turkey

In Turkey, aflatoxin limits are governed by the Turkish Food Codex Regulation on Mycotoxins (2011/29), which aligns with EU standards by setting maximum limits at 2.0 μg/kg for Aflatoxin B_1 and 4.0 μg/kg for total aflatoxins. These limits are strictly monitored by the Ministry of Agriculture and Forestry and the Ministry of Trade. Sampling, testing, and inspection procedures for export products are carried out in compliance with international standards (TGK, 2021). Turkey also places emphasis on producer education, improved storage conditions, and the dissemination of biological control methods. However, implementation challenges among small-scale producers occasionally hinder export performance.

6.5. Global Variation in Aflatoxin Standards

Globally, aflatoxin limits vary by country. For instance, China enforces a 10 μg/kg limit for total aflatoxins in nuts, whereas Japan applies a 10 μg/kg limit solely for Aflatoxin B_1. India permits up to 30 μg/kg for total aflatoxins. These discrepancies necessitate additional trade precautions such as pre-shipment analysis and increase the risk of border rejections for exporting countries (FAO/WHO, 2018).

6.6. Role of Codex and Global Food Safety Systems

At the international level, the Codex Alimentarius Commission, established by FAO and WHO, sets global food safety standards and provides a foundational framework for aflatoxin regulation. While Codex recommends a total aflatoxin limit of 10 μg/kg for tree nuts, this limit is not binding and countries may enforce stricter standards based on public health and trade needs. Additionally, food safety management systems such as the Food Safety Modernization Act (FSMA), ISO 22000, and HACCP play vital roles in guiding producers toward effective aflatoxin risk management (Codex Alimentarius Commission, 2001).

7. CURRENT AND FUTURE APPROACHES IN POST-HARVEST AFLATOXİN MANAGEMENT

Aflatoxin contamination in tree nuts is not solely limited to the production and harvest stages but continues to pose risks during post-harvest handling. Storage conditions, processing techniques, transportation, and marketing all play pivotal roles in controlling aflatoxin formation. Therefore, aflatoxin management requires an interdisciplinary approach, with integrated and sustainable systems being essential for ensuring food safety.

7.1. Environmental Factors and Storage Conditions

Temperature and humidity are the most critical environmental factors influencing aflatoxin development during harvest and storage. The optimal conditions for Aspergillus flavus growth are relative humidity above 80% and temperatures between 25–35 °C (Pitt & Hocking, 2009). Thus, harvested nuts should be dried to a moisture content of 6–8% and stored under moisture-proof conditions (Magan et al., 2003). While traditional sack storage is common, inadequate humidity control can significantly increase aflatoxin risk. Vacuum packaging, controlled atmosphere storage, and moisture-absorbing desiccants are increasingly adopted as effective alternatives (Kabak, 2009). Additionally, extended exposure of harvested nuts to open air or poorly ventilated environments facilitates Aspergillus spore activation and toxin formation (Waliyar et al., 2015).

7.2. Physical and Chemical Detoxification Methods

Complete elimination of aflatoxins from food is extremely challenging; thus, research into physical and chemical detoxification techniques is ongoing. These methods must preserve the nutritional quality of the product and ensure that resulting compounds are non-toxic. Physical methods such as manual sorting, optical sorting machines, and density-based separation techniques are highly effective at removing contaminated kernels. Color sorting systems are particularly useful, as aflatoxin-contaminated nuts often exhibit discoloration (Kaushik et al., 2009).

Among chemical methods, ozonation, ammoniation, and treatments with organic acids are commonly studied. Ozone, a powerful oxidizing agent, can degrade aflatoxins but must be carefully controlled to avoid compromising product quality (Proctor et al., 2004). Ammoniation is accepted in some countries (e.g., for animal feed in the U.S.) but remains restricted or banned in foods intended for human consumption.

7.3. Biological Control Strategies

Biological control is among the most promising strategies for aflatoxin mitigation. Application of non-toxigenic A. flavus strains to the field aims to outcompete toxigenic strains through competitive exclusion (Cotty et al., 2007). This approach modifies the native microbial flora by giving a competitive advantage to non-toxigenic strains. Commercial biocontrol products such as "Afla-Guard®" and "AflaSafe®" have been successfully used in crops like peanuts, maize, almonds, and hazelnuts (Bandyopadhyay et al., 2016). In Turkey, ongoing research focuses on using indigenous non-toxic Aspergillus strains to develop preventive programs (Yentür & Atasoy, 2020).

7.4. Biotechnological Innovations and Molecular Approaches

Advances in biotechnology have enabled the genetic-level investigation of aflatoxin biosynthesis pathways, paving the way for new control strategies. Studies on gene clusters such as aflR, aflD, and aflM have supported the development of RNA-based techniques that suppress or knock down gene expression (Yu, 2012). Moreover, the use of CRISPR-Cas systems to genetically modify aflatoxin-producing strains holds great promise for direct suppression of toxin biosynthesis. While these technologies are currently at the laboratory stage, their integration into field applications is anticipated in the near future (Amaike & Keller, 2011).

7.5. Integrated and Sustainable Management Systems

Sustainable food safety requires a comprehensive approach that goes beyond short-term interventions. This includes farmer education, post-harvest supply chain management, climate-driven early warning systems, and consumer awareness initiatives. Digital agriculture tools, smart sensor systems, and blockchain-based traceability platforms enhance the security and transparency of the food supply chain from production to consumption (Kumar et al., 2008). Ultimately, a systematic structure involving the active participation of all stakeholders is essential for minimizing aflatoxin risks in the long term.

8. CONCLUSION AND RECOMMENDATIONS

Tree nuts occupy a pivotal role in agricultural production owing to their substantial nutritional value and economic significance. Nevertheless, aflatoxin contamination constitutes one of the most severe threats to food safety in these commodities. Aflatoxins, mycotoxins produced by toxigenic fungi such as Aspergillus flavus and Aspergillus parasiticus, exert carcinogenic, hepatotoxic, mutagenic, and immunosuppressive effects on human health (IARC, 2012). The risk of aflatoxin formation is markedly elevated under warm and humid climatic conditions, presenting a significant hazard throughout the entire supply chain from production to consumption.

This study comprehensively addressed the biosynthesis of aflatoxins, contributory factors to contamination, susceptibility of tree nuts to aflatoxin accumulation, analytical detection methodologies, and postharvest management strategies. The findings underscore that environmental parameters, particularly temperature and relative humidity, serve as critical determinants in aflatoxin biosynthesis. Suboptimal postharvest drying, prolonged storage under elevated moisture conditions, and inadequate packaging methodologies were identified as factors that exacerbate aflatoxin proliferation (Magan et al., 2003; Pitt & Hocking, 2009). These insights highlight the imperative for comprehensive education of producers and the implementation of integrated management frameworks spanning the entire production-to-consumption continuum.

Analytical techniques for aflatoxin detection are indispensable for safeguarding food safety. High-sensitivity chromatographic methods, including HPLC and LC-MS/MS, alongside rapid immunoassay-based techniques such as ELISA, are prevalently employed in both production monitoring and regulatory compliance. Nevertheless, the harmonization and standardization of these methodologies according to international protocols, the global alignment of threshold limits, and the development of cost-effective, portable detection systems remain crucial to facilitate on-site application and enforcement (Shephard, 2008; Turner et al., 2009).

Biological control strategies have recently emerged as a promising avenue for aflatoxin mitigation. The deployment of non-toxigenic Aspergillus strains to competitively exclude toxigenic counterparts provides an ecologically sustainable and environmentally benign alternative to conventional chemical approaches (Cotty et al., 2007; Bandyopadhyay et al., 2016). However, for effective implementation, these biocontrol agents require rigorous field-scale validation tailored to regional agroclimatic conditions and specific crop genotypes.

In light of the foregoing, integrated management systems represent the most efficacious approach for aflatoxin control. Such systems encompass a multifaceted paradigm incorporating preharvest agricultural practices, postharvest hygiene, optimal storage conditions, sensitive analytical monitoring, and stringent regulatory frameworks. Concurrently, the enhancement of producer training, consumer awareness initiatives, and the reinforcement of state-sponsored surveillance mechanisms are indispensable components of a sustainable and effective aflatoxin control strategy (FAO, 2018).

From a policy and operational perspective, it is imperative to intensify local-level capacity building, augment the dissemination of knowledge pertinent to aflatoxin prevention, and promulgate good agricultural practices. Furthermore, the establishment of early warning systems integrating climatic data and pathogen epidemiology, alongside the development of comprehensive risk mapping, constitutes the foundation of a proactive aflatoxin management approach. Expanding analytical monitoring infrastructure, particularly in production regions, and improving accessibility for smallholder producers are essential to enhance field-level efficacy. Harmonization of national legislation with international standards, coupled with periodic revision of maximum permissible aflatoxin levels based on emerging scientific evidence, is of paramount importance. Finally, bolstering research and development endeavors aimed at the innovation and commercialization of indigenous biocontrol agents, nanotechnology-based detection platforms, and environmentally sustainable detoxification methodologies will significantly advance the long-term mitigation of aflatoxin contamination.

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CHAPTER 17

CURRENT APPROACHES ON COLD TOLERANCE IN OLIVE (*Olea europaea* L.)

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INTRODUCTION

The olive tree (*Olea europaea* L.) is a xerophytic, evergreen species that has been cultivated in the Mediterranean region for thousands of years (Sanzani et al., 2012; Petruccelli et al., 2022; Gladysheva-Azgari et al., 2025) and is of significant agronomic, economic and cultural importance, playing a key role in shaping the ecological and historical landscape. Olive cultivation is spread over about 11.14 million hectares worldwide, predominantly in more than 40 Mediterranean countries, yielding about 20.3 million tons of olives (FAOSTAT, 2023).

Olive production includes around 3.18 million tons of table olives and 3.38 million tons of olive oil, with Spain, Italy, Greece, and Türkiye being the leading producers, collectively responsible for nearly 95% of global output (FAOSTAT, 2023; IOC, 2024). Furthermore, preliminary data from the International Olive Oil Council (IOC) and the European Commission project a 23% increase in global olive oil production for the 2024/25 season compared to the previous year (ISMEA, 2024).

In recent years, the growing demand for higher-quality and greater quantities of olive oil has led olive cultivation to expand beyond its traditional area in the Mediterranean basin to higher latitudes (Mancuso, 2000; Rahemi et al., 2016) and to gain importance in countries such as Japan, the United States, Australia, China, as well as some countries in South America and South Africa (Rugini et al., 2011). As olive cultivation expands into regions outside its native habitat, which are often exposed to more extreme thermal conditions, assessing the resilience of cultivars to cold and other abiotic stresses has become essential for sustainable production. This is particularly important because exposure to low minimum temperatures—particularly during winter and early spring—remains one of the main limiting factors for successful olive growth in higher latitudes (Rahemi et al., 2016).

Temperature is a fundamental climatic factor governing the distribution of plant species because plants depend on an optimal thermal range to support their growth and development. When temperatures fall outside the optimal range, essential physiological processes in plants slow down or cease entirely, with the severity of these effects influenced by the cultivar, the developmental stage, organ type, and both the rate and duration of exposure to low temperatures (Mancuso, 2000; Wahid et al., 2007; Al-Saif et al., 2024).

Drought and low-temperature exposure are among the primary abiotic constraints affecting the viability and productivity of olive cultivation (Mougiou et al., 2020). This temperature sensitivity of the olive tree largely explains its density in the Mediterranean basin, where the climate is compatible with its thermal requirements.

Among subtropical fruit species, the olive is considered one of the most frost tolerant species; however, olive cultivars may differ in their cold resistance due to variations in the survival capacity of specific organs and tissues (Bartolozzi and Fontanazza, 1999). Numerous studies using various methodologies have been conducted to assess the frost resistance of different olive (*Olea europaea* L.) cultivars.

Understanding the cold tolerance of these varieties is crucial, particularly in the context of increasingly unpredictable and extreme environmental conditions caused by climate change. An accurate assessment of frost resistance can help mitigate the adverse impacts of low temperatures on olive production, ensuring crop sustainability and resilience.

This review comprehensively examines frost tolerance in olive trees, exploring the physiological and biochemical mechanisms underlying cold resistance, as well as the methods used to assess it, genetic variability among cultivars, and strategies aimed at enhancing tolerance to low temperatures. In addition, it highlights current experimental approaches and identifies frost-tolerant olive cultivars reported in recent studies, providing valuable insights for breeders, growers, and researchers working to improve cold resilience in olive cultivation.

THE IMPACT OF LOW TEMPERATURES ON OLIVE TREE PHYSIOLOGY AND SURVIVAL

The degree of resistance to low temperature, also known as cold hardiness, varies among plant species and plays a crucial role in determining their geographical distribution. In the literature, low-temperature stress is generally categorized into chilling stress (0°C to 15°C) and freezing stress (below 0°C) (Gladysheva-Azgari, 2025), both of which can cause damage depending on plant sensitivity and acclimation level (Larcher, 1985; D'Angeli et al., 2003).

The survival of a species at temperatures below freezing is largely influenced by its potential to adapt to cold conditions. The cold acclimation process involves acquiring tolerance to freezing through gradual exposure to decreasing temperatures that remain above the freezing point (Guy, 1990; D'Angeli et al., 2003).

Cold acclimation, triggered by shorter day lengths and decreasing fall temperatures, increases the freezing tolerance of olive trees through a range of physiological and molecular responses. These include changes in gene expression, especially those related to stress proteins, changes in photosynthetic capacity, and adjustments in membrane lipid and protein. In addition, cold acclimation promotes the accumulation of soluble sugars, proline, and stress-associated proteins, which regulate ice crystal formation, maintain osmotic balance, preserve cell turgor, and strengthen dehydration resistance (Ershadi et al., 2016; Al-Saif et al., 2024).

In addition, low temperature stress negatively affects physiological parameters such as relative water content (RWC), photosynthetic pigment levels and stomatal function in olive trees. At the cellular level, membrane integrity is compromised due to the accumulation of reactive oxygen species (ROS), leading to lipid peroxidation, protein degradation and metabolic deterioration. To counteract these effects, olive trees mobilize enzymatic (superoxide dismutase, catalase, peroxidase) and non-enzymatic (phenolic compounds, ascorbic acid, proline) antioxidant defense mechanisms (Ortega-García and Peragón 2009; Gubanova and Paliy, 2020; Gladysheva-Azgari et al., 2025).

A critical limiting factor for olive cultivation is the minimum temperature experienced in winter and early spring (Bartolozzi and Fontanazza, 1999). Olive trees thrive at optimal temperatures between 20 and 30 °C. Metabolic processes such as respiration and photosynthesis slow down significantly at 7.5–12.5 °C, while exposure to freezing conditions, especially below −10 °C, can lead to severe tissue damage or even plant death (Sanzani et al., 2012; Petruccelli et al., 2022). Temperatures below −12 °C can compromise the physiological integrity of olive trees, often resulting in irreversible damage that jeopardizes their survival (Gomez del Campo and Barranco, 2005; Rahemi et al., 2016).

The severity of frost damage in olive trees can be classified into three categories, depending on the phenological stage of the plant and the season in which the frost event occurs. Slight damage is observed on the leaves and one-year-old shoots at temperatures between 0°C and −3°C, while moderate frost, occurring between −6°C and −7°C, affects almost all parts of the plant. Severe frost can threaten the entire plant at temperatures between −12°C to below −18°C (Larcher, 1985; Fiorino and Mancuso, 2000; Sanzani et al., 2012; Rahemi et al., 2016; Petruccelli et al., 2022).

The frost resistance levels of olive organs are ranked from highest to lowest as roots, leaves, shoots, and buds (Fig. 1) (Mancuso, 2000; Gomez del Campo and Barranco, 2005). Due to their underground position, roots are almost never affected by frost. In contrast, leaves exhibit transverse downward curling, chlorotic spots, and brownish discoloration, often accompanied by partial or complete desiccation (Denney et al., 1993; Pezzarossa, 1985; Gomez del Campo and Barranco, 2005). The apical regions of shoots are particularly vulnerable; their damage leads to a shrubby growth pattern as lower buds sprout during the following spring. Frost-affected buds produce fewer inflorescences and lead to morphologically deformed leaves (Connell et al., 1990; Gomez del Campo and Barranco, 2005). In the trunk and branches, reduced bark elasticity lead to in cracking, discoloration occurs, and exudates are secreted by cortical tissues (Denney et al., 1993; Bini et al., 1987; Gomez del Campo and Barranco, 2005). These effects may be compounded by bacterial infiltration through frost-induced wounds (Tombesi, 1986; Gomez del Campo and Barranco, 2005). Fruits can also be affected, with epidermal wrinkling and brownish discoloration on the peduncles, with potential reductions in oil quality and content (Navarro and Parra, 2004; Gomez del Campo and Barranco, 2005). Frost damage also increases susceptibility to bacterial diseases such as olive knots, caused by *Pseudomonas savastanoi* pv. *Savastanoi* (Zucchini, 2024).

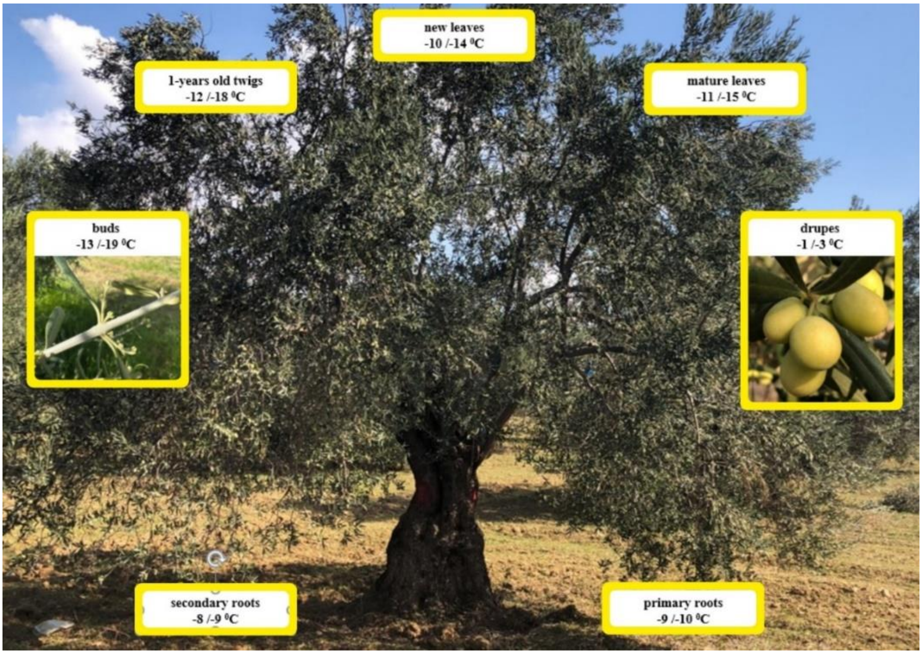

Figure 1. Freezing temperatures for different organs of the olive tree (Graniti et al., 2011; Sanzani et al., 2012; Petruccelli et al., 2022).

In brief, low temperature stress significantly jeopardizes olive tree physiology, with damage severity varying according to organ sensitivity and environmental conditions. Understanding these responses is essential for improving frost management and cultivar selection.

METHODS FOR ASSESSING COLD TOLERANCE IN OLIVE TREES

The assessment of cold tolerance in olive (*Olea europaea* L.) trees has been an important focus in plant physiology and breeding studies, particularly in light of increasingly unpredictable climatic conditions. Over the years, a wide variety of methods have been developed and applied, primarily categorized as morphological, physiological, biochemical, and molecular assessments. These methods have been employed both independently and in combination, depending on the objectives of the study. While some investigations have assessed the response of different olive cultivars following natural frost events, others have conducted controlled freezing experiments under laboratory or greenhouse conditions.

Natural Frost Event-Based Evaluation Techniques

Several studies have investigated cold tolerance in olive cultivars through natural frost events using a combination of morphological evaluations, visual scoring, and recovery assessments. The most commonly employed approach involves visual scoring of frost damage symptoms such as defoliation, bark splitting, and shoot dieback, conducted weeks or months after frost events. These qualitative assessments are often supported by quantitative measurements such as dry matter content of shoots and leaves to obtain a more objective estimation of frost damage.

In Spain, Gómez del Campo and Barranco (2005) assessed frost damage in one-year-old trees of ten cultivars in Madrid after temperatures dropped to -6.5°C and -10.5°C. They combined visual injury scoring with dry matter quantification and found that visual scoring often overestimated damage. They also reported a significant positive correlation between shoot frost resistance and the capacity for vegetative resprouting, emphasizing the importance of regrowth potential as an indicator of frost tolerance.

In Italy, Lodolini et al. (2016) evaluated 24 olive cultivars in Central Italy following a February 2012 frost event. Their methodology combined visual scoring of defoliation and bark splitting with longitudinal monitoring of vegetative resprouting during subsequent growing seasons. This approach provided practical information for cultivar selection in colder climates, distinguishing cultivars not only according to initial frost damage but also according to their ability to recover and survive. Subsequently, Lodolini et al. (2022) extended this research by comparing frost damage and recovery in eleven cultivars following two freezing events in 2012 and 2018. Utilizing standardized visual indexes for damage and resprouting in different tree sections, they emphasized that frost sensitivity depends on factors such as tree age, physiological status, and environmental conditions, highlighting the need for multi-year, multi-event evaluations.

Keramatlou et al. (2019) focused on identifying and selecting frost-resistant trees following a severe frost event in Golestan Province, Iran in 2016. In their study, they applied detailed morphological characterization including canopy and trunk measurements, leaf defoliation rates, and bark splitting observations to select trees with superior cold resilience. Building on this, Keramatlou et al. (2023) conducted a large-scale field evaluation of 218 mature olive trees exposed to repeated frost events Using 19 morpho-agronomic traits combined with genetic profiling and image-based analyses, they selected 45 genotypes exhibiting stable frost tolerance characterized by the absence of bark splitting and leaf drop. These cold-tolerant genotypes also displayed promising oil content, indicating their suitability for cultivation in frost-prone regions.

Additionally, Saadati et al. (2020) examined physiological changes in leaves of three olive cultivars during cold acclimation and de-acclimation stages under natural cold conditions in Isfahan, Iran. This region experiences relatively long, warm summers, but winters with minimum temperatures that can drop below −10°C, causing significant damage to olive trees. During the study period, average temperatures ranged from 18.15°C in November to as low as 5.6°C in February, with recorded minimum temperatures falling below freezing. These natural cold conditions provided a realistic setting for assessing cold tolerance in three olive cultivars through sampling at multiple time points during cold acclimation and de-acclimation phases

The methods employed in these studies demonstrate the effective integration of visual damage assessment, quantitative morphological measurements, genetic profiling, and recovery monitoring as comprehensive approaches for evaluating cold tolerance in olive trees under field conditions. These methodologies provide a robust framework for identifying and selecting cultivars capable of withstanding frost stress, which is critical for sustaining olive production in regions facing increasing climatic variability.

Controlled Freezing Temperature Treatments in Olive Cold Tolerance Studies

Cold tolerance in olive cultivars has been evaluated using a wide range of freezing temperatures, from mild chilling (4 °C) to extreme subzero stress (−25 °C) under controlled conditions. Findings from these studies consistently highlight the critical influence of temperature severity on cultivar responses.

In several experiments, temperature treatments including 4, −5, −10, and −20 °C (Cansev et al., 2011), as well as −5, −10, −15, and −20 °C applied for 10 hours (Hashempour et al., 2014a, 2014b), were employed to compare cold-acclimated and non-acclimated tissues. Similarly, Rahemi et al. (2016) utilized 0, −6, −12, and −18 °C, whereas Rezaei et al. (2013) investigated a wider temperature range from 10 °C to −20 °C, identifying cultivar-specific thresholds for physiological disruption.

More severe freezing treatments were applied in studies such as Saadati et al. (2019), where temperatures ranging from 0 °C to −25 °C were used to determine LT_{50} values among seven cultivars. Vafadar et al. (2024) evaluated freezing tolerance under both laboratory and field conditions by applying temperatures from 0 to −21 °C, while Mete et al. (2023) exposed leaves to +4 °C (control) down to −20 °C, revealing distinct genetic variation in cold response.

In transcriptomic and molecular studies, Gladysheva-Azgari et al. (2025) exposed cold-tolerant and susceptible cultivars to −7 and −12 °C to, while Forcada et al. (2016) subjected samples to −4 °C for 98 hours, indicating even moderate freezing can trigger deep regulatory responses.

Eris et al. (2015) monitored seasonal changes in cold hardiness by exposing samples to 4, −5, −10, and −20 °C, capturing annual variation in cold acclimation. In screening studies, Bartolozzi and Fontanazza (1999) tested −8, −10, −12, and −14 °C, while Valverde et al. (2024) proposed −10 °C for 30 minutes (followed by a recovery period) as an effective standard for leaf-level damage assessment. Additionally, they identified −7 °C as critical for potted olive plants.

Overall, the literature indicates that olive cultivars exhibit varying responses across a temperature range spanning from mild chilling to extreme freezing (−25 °C). Consequently, cold tolerance assessments under controlled conditions are highly influenced by both the severity and duration of temperature exposure.

Visual and Morphological Evaluation Methods

Visual and morphological assessments are widely employed in cold tolerance studies due to their practicality and ability to capture phenotypic responses in a straightforward and non-destructive manner. These methods typically include scoring parameters such as leaf defoliation, bark splitting, canopy damage, and vegetative resprouting following exposure to freezing conditions. In numerous studies, visual scoring has been complemented by quantitative morphological measurements—including tree height, canopy diameter, trunk cross-sectional area, and internode length—enabling a more objective interpretation of stress responses (Keramatlou et al., 2019; Keramatlou et al., 2023).

Field evaluations during natural frost events have demonstrated that these parameters can reliably distinguish between tolerant and sensitive genotypes when applied systematically across multiple time points. For instance, Lodolini et al. (2016, 2022) implemented visual indexes from 0 (no damage) to 3 or 4 (maximum damage) to evaluate defoliation and bark injury, followed by scoring of resprouting in apical, central, and basal sections of the tree. These scores showed strong correlations with cultivar resilience and recovery capacity over consecutive years. Similarly, Valverde et al. (2024) proposed a standardized approach using detached leaves and potted plantlets under controlled conditions (−10 °C for leaves; −7 °C for plantlets), followed by a recovery phase, demonstrating clear visual differences among cultivars. Importantly, this method was validated as both sensitive and reproducible, and it effectively distinguished between acclimated and non-acclimated tissues.

In field evaluations, metrics such as shoot length, tree height, and leaf area have also been employed to assess cold damage and regrowth potential. Al-Saif et al. (2024) systematically measured these traits across multiple cultivars, revealing distinct varietal responses to night frost events. Their results confirmed that visual and morphological traits effectively reflect broader physiological and reproductive resilience under cold stress.

The use of visual assessment parameters is further supported by their strong correlation with underlying physiological and biochemical traits, such as antioxidant activity and membrane integrity, although these are often evaluated in parallel studies. Despite some limitations in subjective interpretation, visual methods remain a cost-effective and scalable approach, particularly when integrated with quantitative morphological criteria and applied over multiple seasons.

Physiological Assessments in Cold Stress Studies

Relative Water Content (RWC) and Leaf Water Potential

RWC and leaf water potential are commonly used to assess cold tolerance in olive due to their effectiveness in reflecting plant water status under stress conditions. These parameters help indicate the plant’s ability to maintain hydration and turgor during freezing, offering a reliable perspective on stress-induced dehydration.

A-Saif et al. (2024) calculated RWC in four olive cultivars by measuring fresh, saturated, and dry leaf masses, and found cultivar-specific differences under night frost conditions, with 'Coratina' and 'Koroneiki' showing better water retention. Saadati et al. (2019) compared seven cultivars and observed that tolerant types had lower relative water loss than sensitive ones, highlighting a correlation between RWC and freezing resistance. Similarly, Arias et al. (2021) reported that water deficits applied before winter reduced leaf water potential and enhanced cold hardiness across cultivars, showing that pre-stress conditioning influences physiological responses.

Stomatal Conductance

Stomatal conductance has been employed to assess cold-induced dehydration and its relation to frost tolerance in olive. Under soil chilling conditions, significant reductions in leaf conductance were observed in some genotypes, indicating timely stomatal closure as a protective mechanism against water loss (Pérez-López et al., 2010). Comparative analyses revealed that cultivars with lower stomatal density generally exhibited higher freezing tolerance, suggesting an inverse correlation between stomatal opening potential and cold resilience (Rehami et al., 2016; Saadati et al., 2019). Similarly, Dadras et al. (2024) identified stomatal conductance, alongside proline content, as a key physiological trait positively associated with cold tolerance, highlighting its value as a practical indicator for screening cold-hardy olive genotypes. These findings imply that measuring stomatal conductance and density can serve as practical physiological indicators in screening for cold-tolerant olive genotypes.

Chlorophyll Content (Chl a, Chl b) and Carotenoids

Chlorophyll content, including chlorophyll a, chlorophyll b, and carotenoids, is a critical physiological indicator for assessing the impact of cold stress on olive trees. In the study by Al-Saif et al. (2024), pigments were extracted from fresh leaves using acetone homogenization followed by spectrophotometric measurement, enabling precise quantification of photosynthetic pigments. Similarly, Rezai et al. (2013) assessed chlorophyll levels in olive cultivars exposed to various low temperatures, revealing differential pigment stability associated with cold tolerance. Monitoring these pigments provides valuable insights how cold stress affects photosynthetic capacity and overall plant health in olives.

Electrolyte - Ion (EL) Leakage Measurement

Electrolyte leakage (EL) is one of the most widely used physiological methods to assess cold-induced membrane damage in olive leaves and shoots. This technique evaluates cell membrane stability by measuring the amount of ions that leak out as a result of membrane injury under low-temperature stress. In several studies, leaf or shoot samples were exposed to varying freezing temperatures, and electrical conductivity was measured both before and after autoclaving to determine the extent of damage (Azzarello et al., 2009; Mete et al., 2023). Results consistently revealed significant differences among cultivars, with more tolerant genotypes showing lower EL values (Bartolozzi and Fontanazza, 1999; Rehami et al., 2016). Moreover, EL data has often been interpreted water relations, such as dehydration caused by impaired stomatal function, offering insights into cultivar-specific responses to cold (Pérez-López et al., 2010). Due to its sensitivity, reproducibility, and ease of application, EL measurement has become a reliable screening tool for evaluating cold tolerance in olive.

LT_{50} (Lethal Temperature 50%) Measurement

LT_{50}, or the lethal temperature at which 50% of tissue injury occurs, is a widely used physiological parameter for quantifying cold tolerance in olive trees. It represents the temperature threshold where half of the plant cells sustain irreversible damage under freezing stress. Mete et al. (2023) estimated LT_{50} using ion leakage tests to screen 40 Turkish olive cultivars under different temperature treatments and seasons, revealing significant genetic and seasonal variability in frost tolerance. Similarly, Eris et al. (2015) monitored LT_{50} changes monthly in 'Gemlik' olive shoots during two consecutive years, showing that cold hardiness increased during autumn and winter in parallel with rises in total soluble sugars and proteins. Rezaei and Rohani (2023) compared multiple nonlinear regression models for calculating LT_{50} based on electrolyte leakage and tetrazolium tests, highlighting models effective for different cultivars and dormancy stages. Additionally, Arias et al. (2021) demonstrated that LT_{50} values significantly decreased following cold acclimation and water deficit treatments, emphasizing the importance of environmental conditions on freezing resistance and growth trade-offs. Overall, LT_{50} measurement is a crucial, quantitative method that integrates biochemical and physiological responses, offering valuable insights into cultivar selection and cold stress management in olive cultivation.

Biochemical Markers of Cold Response

Lipid Peroxidation (MDA)

Lipid peroxidation is one of the earliest biochemical responses to cold-induced oxidative stress, and is commonly assessed by measuring malondialdehyde (MDA) content. Several studies have demonstrated that MDA levels increase in olive leaves under freezing conditions, correlating with the severity of membrane damage. For instance, in ‘Fishomi’ and ‘Roughani’ cultivars, MDA content rose significantly under -5 to -20°C conditions, with ‘Roughani’ exhibiting higher values, indicating its greater susceptibility (Hashempour et al., 2014a). Similarly, Saadati et al. (2020) observed lower MDA levels in the cold-tolerant ‘Amphisis’ cultivar during cold acclimation, suggesting enhanced membrane stability. In another study, exposure to low temperatures led to significant increase in MDA in olive cv. ‘Zard’, while salicylic acid pretreatment reduced its accumulation, indicating a protective role (Hashempour et al., 2014b). Rezaei et al. (2013) reported that MDA levels increased in both ‘Frantoio’ and ‘Sevillana’ cultivars under cold stress, with lower accumulation in the more tolerant ‘Frantoio’. Taken together, MDA quantification is a reliable biochemical marker of cold-induced oxidative membrane damage in olive.

Total Soluble Sugars and Proteins (TSS, TSP)

The accumulation of total soluble sugars (TSS) and proteins (TSP) plays a crucial role in osmotic adjustment and cryoprotection under cold stress. Eris et al. (2015) reported a seasonal increase in TSS and TSP in olive cv. ‘Gemlik’ during autumn and winter, which paralleled enhanced cold hardiness. Similarly, Gulen et al. (2009) demonstrated that cold acclimation led to significant increases in reducing sugars and sucrose, particularly in cold-tolerant cultivars. In that study, sucrose accumulation was more prominent than reducing sugars, indicating its central role in cold adaptation. Additionally, Saadati et al. (2020) observed higher TSP levels in cold-tolerant ‘Amphisis’, which declined during de-acclimation phase. Total protein changes in response to low temperatures were also cultivar-dependent: ‘Frantoio’ maintained its protein levels better than ‘Sevillana’ under -10°C (Rezaei et al., 2013). Overall, TSS and TSP serve as biochemical indicators of cold-induced metabolic adjustments, with higher levels correlating with increased freezing tolerance.

Proline Content

Proline is a key osmoprotectant and antioxidant, contributing to cellular stabilization under cold stress. Hashempour et al. (2014a) reported significantly higher proline accumulation in the cold-tolerant ‘Fishomi’ compared to ‘Roughani’, which was negatively correlated with freezing injury. Similarly, Saadati et al. (2020) recorded increased proline levels during cold acclimation in the more tolerant ‘Amphisis’ cultivar. Rahemi et al. (2016) also observed elevated proline content in the freezing-tolerant cultivars ‘Zard’ and ‘Dehghan’. In contrast, Hashempour et al. (2014b) found that proline levels declined under freezing conditions, however, treatment with 1 mM salicylic acid effectively restored its concentration, enhancing tolerance in cv. ‘Zard’.

Dadras et al. (2024) utilized genotype-by-trait (GT) biplot analysis to identify cold-tolerant olive genotypes based on multiple biochemical traits. Cultivars such as ‘Grossane’, ‘Abou-satl’, ‘Zard’, and ‘Picual’ exhibited high proline levels, indicating superior frost resistance.

Taken together, these findings confirm that proline is a consistent biochemical marker associated with cold tolerance mechanisms in olive.

Total Phenolic and Flavonoid Content

Phenolic compounds and flavonoids are secondary metabolites with antioxidant properties that mitigate cold-induced oxidative stress. Al-Saif et al. (2024) reported higher total phenolic and flavonoid contents in cold-tolerant cultivars such as ‘Coratina’ and ‘Koroneiki’, which correlated with their better biochemical performance under night frost conditions. Cansev et al. (2011) observed that cold-acclimated leaves of ‘Gemlik’ exhibited increased phenolic contents, particularly following -10°C and -20°C treatments, which were positively correlated with antioxidant capacity. Rahemi et al. (2016) also noted elevated phenolic levels in cold-tolerant cultivars ‘Zard’ and ‘Dehghan’. In addition, Ortega-García and Peragón (2009) found increased concentrations of oleuropein—a key phenolic compound—in moderately and severely cold-stressed leaves of cv. 'Picual', highlighting its potential role in defense mechanisms. These e findings collectively suggest that phenolic accumulation serves as a valuable biochemical marker associated with cold response in olive.

Antioxidant Enzyme Activities (SOD, CAT, APX, POD, POX, PPO)

Antioxidant enzymes play a crucial role in the plant's defense system against reactive oxygen species (ROS) generated during cold stress. Hashempour et al. (2014a) reported increased activities of catalase (CAT), ascorbate peroxidase (APX), peroxidase (POD), and polyphenol oxidase (PPO) in 'Fishomi' cultivar, which correlated with its lower MDA levels and ion leakage. In contrast, 'Roughani' cultivar exhibited higher SOD activity. Hashempour et al. (2014b) demonstrated that salicylic acid treatment enhanced the activities of these enzymes, contributing to freezing tolerance in cv. 'Zard'. Cansev et al. (2009) observed higher CAT and APX activities in cold-acclimated cultivars, particularly Domat and Lecquest, and linked this to the expression of 43 kDa dehydrin proteins. Similarly, Cansev et al. (2011) found seasonal variation in CAT and APX activities in cv. 'Gemlik', with higher values in autumn and winter, coinciding with lower injury levels. Ortega-García and Peragón (2009) reported that PPO activity increased significantly after cold stress in cv. 'Picual', with the highest activity observed in severely stressed samples. Furthermore, Saadati et al. (2020) showed enhanced activities of APX, CAT, POD, and SOD during acclimation in the cold-tolerant 'Amphisis', supporting their relevance as key markers of cold tolerance.

Genetic Studies on Cold Tolerance in Olive

Recent advances in molecular research have significantly enhanced our understanding of the genetic mechanisms underlying cold tolerance in olive (*Olea europaea* L.). Contemporary studies employing transcriptomic analyses and QTL (Quantitative Trait Loci) mapping have identified key regulatory genes and molecular pathways activated during cold stress.

For instance, Gladysheva-Azgari et al. (2025) analyzed the transcriptional responses of cold-tolerant and cold-susceptible cultivars exposed to moderate (−7 °C) and severe (−12 °C) freezing conditions. Their study revealed distinct gene expression profiles associated with photosynthetic recovery and cell membrane stability, identifying two differentially expressed genes (DEGs) commonly upregulated across all cultivars during early freezing.

Similarly, Guerra et al. (2015) utilized RNA-Seq to investigate cold acclimation in cv. ‘Leccino’, identifying 5,464 DEGs. These included genes involved in ROS scavenging (e.g., glutathione cycle, flavonoid biosynthesis), osmolyte accumulation (raffinose, trehalose), and cell wall restructuring (callose and lignin biosynthesis), all modulated through ABA signaling.

Hashempour et al. (2019) focused on the expression of fatty acid desaturases (FAD2, FAD6, FAD7) and beta-glucosidase (BGLC) in freezing-tolerant (cv. ‘Fishomi’) and sensitive (cv. ‘Zard’) cultivars. Their results indicated that enhanced expression of these genes in the tolerant cultivar supported membrane fluidity and recovery under cold stress.

Wu et al. (2024) identified a novel regulatory module involving the transcription factor bHLH66 and ELIP1, which was highly induced under chilling in cv. ‘Arbequina’. This interaction was shown to protect photosynthetic capacity, providing new insights into cold adaptation mechanisms.

Complementing these transcriptomic findings, Cetin et al. (2025) performed QTL mapping in a ‘Memecik’ × ‘Uslu’ hybrid population, identifying genomic regions associated with frost tolerance. These markers, distributed across 23 linkage groups corresponding to the olive haploid chromosome number, offer promising tools for marker-assisted breeding.

Overall, these recent molecular studies illuminate the complex genetic architecture of cold tolerance in olive and pave the way for developing more resilient cultivars through precision breeding strategies.

COLD-TOLERANT OLIVE CULTIVARS IDENTIFIED WORLDWIDE

Understanding and improving cold tolerance in olive cultivars is crucial for expanding cultivation into cooler regions and mitigating frost damage. Table 1 summarizes key studies from various countries that have identified cold-tolerant olive cultivars. By integrating physiological, biochemical, and molecular approaches, these studies offer a comprehensive and multidimensional assessment of cold tolerance. Presenting this information together allows for clearer comparisons of resistant varieties across different countries and research approaches.

Table 1. Cold-tolerant olive varieties reported in recent studies

Study	Country / Region	Tested Cultivars / Genotypes	Cold-Tolerant Cultivars Identified	Assessment Method / Criteria
Bartolozzi & Fontanazza (1999)	Italy	9 cultivars and 3 selections, control Frantoio (Tolerant)	‘Bouteillan’, ‘Nostrale di Rigali’	Controlled freezing tests at −8 to −14°C; ion leakage and LT_{50}, visual damage scoring
Gómez del Campo & Barranco (2005)	Spain / Madrid	10 cultivars	‘Cornicabra’, ‘Arbequina’	Natural frost, visual scoring, dry matter, resprouting capacity
Cansev, Gulen & Eris (2009)	Turkey	‘Ascolona’, ‘Domat’, ‘Gemlik’, ‘Hojoblanca’, ‘Lecquest’, ‘Manzanilla’, ‘Meski’, ‘Samanli’, ‘Uslu’	‘Domat’, ‘Lecquest’	Cold acclimated vs non-acclimated leaves; LT_{50} by electrolyte leakage at 4 to −20°C for 12h; antioxidative enzymes and dehydrin protein analysis
Rezaei et al. (2013)	Iran	‘Frantoio’, ‘Sevillana’	‘Frantoio’	Controlled freezing (10 to −20 °C); physiological and biochemical parameters: MDA, total protein, photosynthetic pigments
Rahemi et al. (2016)	Iran	‘Amygdalolelia’, ‘Conservallia’, ‘Dakal’, ‘Shiraz’, ‘Dehghan’, ‘Zard’, ‘Dezful’, ‘Tokhme-Kabki’	‘Zard’, ‘Dehghan’	Controlled freezing (−6 to −18 °C); stomatal density, ionic leakage, starch & proline content, phenolics, sugars
Lodolini et al. (2016)	Italy / Marche	24 cultivars	‘Ascolana dura’, ‘Orbetana’	Natural frost (−10.5 °C); visual scoring (defoliation, bark split), vegetative re-sprouting
Lodolini et al. (2022)	Italy	11 cultivars	Not explicitly stated in the abstract	Multi-year frost events (2012 & 2018), visual scoring (defoliation, bark split); recovery index (resprouting ability)
Keramatlou et al. (2019)	Iran / Golestan	118 trees initially screened	38 unnamed cold-tolerant genotypes	Natural frost, morphological traits (leaf drop, bark splitting), tree vigor
Keramatlou et al. (2023)	Iran / Golestan	218 genotypes	45 stable frost-tolerant genotypes	Field evaluation, 19 morpho-agronomic traits, genetic markers, imaging

Table 1. Cold-tolerant olive varieties reported in recent studies (continued)

Study	Country / Region	Tested Cultivars / Genotypes	Cold-Tolerant Cultivars Identified	Assessment Method / Criteria
Saadati et al. (2018)	Iran	7 cultivars: 'Amphisis', 'Shengeh', 'Conservallia', 'Rashid', others	'Amphisis', 'Shengeh', 'Conservallia'	Freezing at 0 to −25°C; chlorophyll fluorescence (Fv/Fm), soluble carbohydrates, MDA, stomatal density, leaf water content
Saadati et al. (2020)	Iran / Isfahan	'Amphisis', 'Gorgan', 'Manzanilla'	'Amphisis'	Seasonal physiological sampling during cold acclimation and de-acclimation: RWC, Proline, MDA, antioxidant enzymes (SOD, CAT, APX, POD)
Mete et al. (2023)	Turkey	40 registered Turkish cultivars	'Butko', 'Memeli', 'Otur', 'Gemlik', 'Sinop No 5', 'Yün Çelebi', 'Kara Yaprak', 'Satı', 'Sarı Ulak'	Controlled freezing tests, ion leakage (electrical conductivity), LT_{50} estimation
Al-Saif et al. (2024)	Egypt	4 cultivars	'Coratina', 'Koroneiki'	Field study under natural cold; visual observations; RWC, chlorophyll content, total phenolic & flavonoid, enzyme activity (CAT, POD, APX, SOD)
Valverde et al. (2024)	Italy	'Arbequina', 'Arbosana', 'Ascolana Tenera', 'Frantoio', 'Leccino', 'Picual'	'Arbequina'	Lab method: detached leaves at −10°C for 30 min; recovery at 26°C for 24–48 h; potted plantlets at −7°C; acclimated vs non-acclimated conditions
Rezaei and Rohani (2023)	Iran	'Dorsalani', 'Luke', 'Roughani', 'Shangeh', 'Rashid', 'Mission', 'Kawi', 'Abu-Stal', 'Manzanilla', 'Zard'	'Roughani', 'Kawi', 'Zard'	Artificial freezing; electrolyte leakage (EL) and tetrazolium (TZ) tests; LT_{50} and LT_{90} estimated using nonlinear regression models
Dadras et al. (2024)	Iran	16 genotypes including	'Grossane', 'Abou-satl',	Genotype-by-trait biplot analysis based on 15

		'Zard', 'Grossane', 'Abou-satl', 'Picual', 'Konservolia', 'Cornicabra', 'Amin', 'Kaissy', 'Koroneiki', 'Meshkat', 'Direh'	'Zard', 'Picual', 'Konservolia', 'Cornicabra', 'Amin'	physiological and biochemical traits including proline content and stomatal conductance
Gladysheva-Azgari et al. (2025)	Russia (Crimea)	'Nikitskaya-2', 'Tossiyskaya', 'Tiflisskaya' (cold tolerant); 'Leccino', 'Razzo', 'Coreggiolo' (cold susceptible)	'Nikitskaya-2', 'Tossiyskaya', 'Tiflisskaya'	Physiological and biochemical assays; transcriptomic analysis under −7°C and −12°C freezing stress; DEGs profiling; 3 biological replicates per cultivar

CONCLUSIONS

Cold stress continues to be a significant limiting factor for olive (*Olea europaea* L.) cultivation, especially due to increasing climatic variability and unpredictable frost events worldwide. The comprehensive studies reviewed in this paper highlight the diverse approaches used to assess and understand cold tolerance in olive, including morphological, physiological, biochemical, and molecular methods.

Field assessments based on natural frost events, when integrated with controlled freezing experiments, provide complementary information on cultivar-specific responses. Visual and morphological assessments, particularly when performed across multiple seasons, remain practical and cost-effective tools for reliably distinguish tolerant genotypes. Meanwhile, physiological parameters such as relative water content, stomatal conductance, chlorophyll stability, and electrolyte leakage provide quantitative measures of stress at the cellular and tissue levels, allowing more precise screening.

Biochemical markers, including malondialdehyde (MDA), total soluble sugars and proteins, proline content, phenolic and flavonoid compounds, and antioxidant enzyme activities, serve as strong indicators of oxidative damage and defense mechanisms activated during cold exposure. These biochemical responses are often closely correlated with observed phenotypic tolerance, making them valuable tools in both research and breeding programs.

Advances in molecular genetics and transcriptomics have provided valuable insights into the complex gene networks and regulatory pathways involved in cold acclimation and freezing tolerance. Identification of differentially expressed genes, candidate QTLs, and key transcription factors offers promising tools for marker-assisted selection and precision breeding of frost-resistant olive cultivars.

Studies conducted across various regions—including the Mediterranean basin, Iran, Türkiye, Egypt, and Russia—have consistently identified olive cultivars exhibiting superior cold tolerance. These cultivars, validated through integrated physiological and molecular criteria, represent valuable genetic resources for expanding olive cultivation into colder climates and reducing frost-related losses. However, genotype-by-environment interactions highlight the need for local and multi-seasonal validation of cultivar performance under cold stress.

In conclusion, enhancing cold tolerance in olive cultivation demands sustained, multidisciplinary research that integrates long-term field evaluations, biochemical screening, and genomic approaches. These integrated strategies are essential for ensuring the resilience, sustainability, and productivity of olive orchards under increasing climatic uncertainty.

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