COMPENDIUM OF ADVANCES IN AGRICULTURE

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PREFACE

Agriculture has been one of the main sources of human existence throughout history. It is of great importance as nutrition for humans and animals, and basic input to various industries. From the existence of mankind to the present day, it has gone through various stages in terms of production activities. Since the end of the 18th century, the traditional society structure based on agriculture has changed. Rapid population growth, shrinkage of agricultural areas, mechanization in agriculture and the effects of this great transformation on the environment have left agricultural producers in a conundrum. By the end of the 1950s, following the period of the first transformation called "Agriculture 1.0", synthetic pesticides, fertilizers and more effective machinery reduced production costs and thus we entered the "Agriculture 2.0" period, called the Green Revolution. Efficiency increased, thanks to cheap inputs and new tools. With the introduction of GPS signals to everyday use, the "Agriculture 3.0" process started in the 1990s, today this period is more commonly called Precision Agriculture. The impact of the concept of “Agriculture 4.0”, which has left its mark on today, on agriculture is felt more and more. During this period, innovative and effective tools were created to make the agricultural sector more efficient, competitive, environmentally friendly and sustainable. Robots and artificial intelligence started to be used in the agricultural sector and more products could be grown in a faster and healthier way. In the " Agriculture 5.0" model, which emerged as a paradigm shift that aims to direct the focus and purpose of production systems from high profit and market share to social contribution by going beyond the " Agriculture 4.0" model, "Sustainability" is a resource-based philosophy, while meeting the present needs of humanity, while considering the needs of future generations.

Considering that the agricultural sector has a very important responsibility to feed the ever-increasing world population by prioritizing factors such as productivity and sustainability, today's agriculture needs to be planned in a structure that is based on modern technologies and can compete with the world.

Increasing productivity in agriculture is a multi-faceted process which involves, in general, practices such as fertilization, irrigation, fight against diseases and pests, and utilization of machinery and technology, as well as transportation, storage, and marketing. Three dimensions that must be considered simultaneously in ensuring sustainability have been defined as "Economy", "Society" and "Environment", and it is aimed to evolve the current economy-oriented industry model in a way that includes social and environmental effects in decision-making processes.

In the book named “Compendium of Advances in Agriculture”, it has been tried to reveal the developments and changes in agricultural science over the years of agricultural practice. In pace of protecting environment, biological control methods for nematodes and mites, and utilization of bioactive compounds and microorganism in the soil are under spotlight. Increasing oil quality for tending human health and describing commodity quality using non-destructive methods has been attracting attention. As the climate change forces agricultural areas to move previously unconsidered areas such as higher altitudes, we are going to have to change our traditional way of implementing cultural practices. This book was intended as a reference to increase and dispense the scientific knowledge in the selected subjects. We are greatly in debt to all authors in this book for their scientific efforts and contribution.

EDITORS

CHAPTER 1

AGRICULTURE 5.0

Assoc. Prof. M. Tolga ESETLILI[1*], Agr. Eng. Edanur FIRAT[1b]

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INTRODUCTION

Agriculture is one of the oldest and most effective fields that underpin the economic growth of every country. The origin of the word agriculture comes from the Latin word "ager” meaning soil and “culture” meaning cultivation. Agriculture is also recognized as one of the largest employment and income-generating sectors. Compared to ancient times, many technological tools and new techniques are now available in the agricultural sector. Besides these latest techniques, the environmental factor also plays a significant role. Farmers globally face numerous problems related to agriculture, such as the increasing demand for food products and climate change, which, in recent periods, has mostly affected the growth of crops. Moreover, economic pressures on inputs used in agriculture, such as seeds, fertilizers, and pesticides, appear as other major problems. According to the Food and Agriculture Organization (FAO), approximately 70% additional food must be produced to meet the food needs of the increasing population. Therefore, it has now become a requisite to shift to smart farming instead of traditional agricultural practices.

Apart from this, the choice of crops to be grown largely depends on climatic factors such as temperature, humidity, sunlight, moisture, etc. Additionally, the physical properties of soils, such as texture, color, structure, and other soil properties, including pH, lime, salinity, and organic matter content, are also essential. Furthermore, irrigation water, its quality, quantity, and management are of critical importance as well. When addressing the issue globally, one should note that sufficient quantity and quality of water for irrigation purposes is not available, which decreases each year.

At present, to sort out all these challenges, agricultural entrepreneurs and large companies are using smart agriculture techniques that utilize efficient farming methods. Smart agriculture techniques play a key role in increasing crop yields in less time with minimal human intervention, increasing global market connectivity through smart monitoring activities, improving monitoring and weather forecasting of climate parameters to feed different crops, etc. Thus, the production systems used are more cost-effective. Through smart farming and Information Communication Technologies (ICT), farmers can now monitor their fields and easily receive information from anywhere in the world.

Smart agriculture receives relatively more attention for the following substantial reasons.

Over the last few decades, the biggest challenge to traditional farming methods has been climate change. Evaporation, temperature, and drought, as well as the amount, timing, and form of precipitation, always play a dominant role in the productivity of agriculture. These factors also affect the life cycles of plants. Therefore, smart agriculture practices are widely used in response to these issues and challenges. The global share of the smart agriculture market also increases. Therefore, in smart farming, devices contribute a lot to enhancing agriculture productivity, as they are used in monitoring soil quality, soil temperature and moisture, irrigation, and fertilization practices, along with climate data. These applications can be employed not only for plant production but also for animal production. Likewise, they help to monitor the quality of live stocks and crops. To conduct these activities, numerous sensors are used to provide farmers with real-time data on climate, soil, plant growth, diseases, and pests.

The main benefits of the techniques used in smart agriculture can be listed as follows;

1. Efficient water management through sensors
2. Monitoring the soils before, during, and after plant cultivation
3. Real-time monitoring of applications such as fertilization, spraying, and irrigation for proper crop growth
4. Early warning systems for plant diseases
5. Reduction of the labor force, saving time, reliability, and labor cost minimization
6. Increasing crop sales without geographical restrictions

1. TECHNOLOGICAL DEVELOPMENT OF AGRICULTURE

The evolution of agriculture in human history comprises a combination of scientific and technological revolutions and developments leading to increased food production efficiency. When this evolutionary process is evaluated, the technological transformation of agriculture dates back to the early 20th century. In parallel with the industrial ages, agriculture also divides into various periods. The most fundamental feature of the period of the first transformation, called Agriculture 1.0, was experienced was that it had a labor-intensive mode of production with low productivity. By the late 1950s, synthetic pesticides, fertilizers, and more effective machinery reduced production costs, and thus the Agriculture 2.0 era, called the Green Revolution, emerged. Efficiency increased owing to low-cost inputs and novel tools. Agriculture 3.0, or “precision agriculture," began after the introducing tele-mechanical methods based on GPS technology, including satellites, managing agricultural tools. Agriculture 3.0 is characterized by the intensive development of biotechnology and genetic engineering, the differentiation of farm products based on data analysis (including the ones collected through remote sensors), and the optimization of agricultural production through reduced expenses and increased profitability (Baryshnikova, et al., 2022).

In these periods, the widespread use of agricultural machinery, which increased with the industrial revolution, and technological developments such as the green revolution initially increased production; however, over time, they stipulated a modern agricultural revolution as well to make it environmentally sustainable. Agricultural areas have been lost because of soil erosion, deforestation, improper land use, overgrazing, improper crop rotation, and the use of unbalanced fertilizer, along with excessive pesticide use. Due to those factors, the global deterioration of arable land and the decrease in usable water resources over time led to a focus on agriculture as well as on climate change. According to FAO, current agricultural production should increase by 70% by 2050, given the increasing demand for high-quality and environmentally friendly food (FAO, 2022). Similarly, on September 25, 2015, the United Nations (UN) General Assembly approved “Transforming Our World: The 2030 Agenda for Sustainable Development," an action plan for the well-being of people and the planet (UN, 2022). Within the scope of this agenda, 17 Sustainable Development Goals with a completion period from 2015 to 2030 replaced the previously drafted Millennium Development Goals (Martos et al., 2021). In tandem with those initiatives, ensuring global food security and sustainability became strongly correlated with the use of information technologies in agriculture. Post-industrial food systems replaced traditional food systems. A fundamental impetus of agricultural development in the 21st century has been digital transformation.

With the impact of Industry 4.0, the digitalization process in industrial production began affecting the agricultural sector as well. All agricultural machines used in the agricultural production process are now equipped with sensors, and the Internet of Things (IoT) has also entered the agricultural sector, thus ensuring the interconnectedness of machines throughout the entire production process. The concept of smart agriculture, which came into our lives in this period, also described as Agriculture 4.0, is based on the data transfer principle and ensures data concentration in storage systems to bring various terrain data together and analyze it for decision-making. With digitalization, data is collected and analyzed through smart tools. These applications ensure the determination of how much and what kind of fertilizer should be given to which areas, the provision of the minerals and irrigation needed by the plants, and facilitate pest control. They pave the way for producers by indicating the estimated harvest time in detail and in real-time and aim to maximize yield and quality parameters by minimizing inputs and pollution factors compared to traditional methods.

Using smart agriculture technologies and related applications is preferred as it increases the success rate in agricultural decision-making stages owing to the ease of obtaining data and the cost advantages it provides in agricultural production. Smart agriculture platforms are an effective tool for many new users and usage. Smart agriculture demands up-to-date information from production systems, markets, and intermediaries involved in production so as to provide decision-making information for production as well as related strategic and managerial issues (Pivoto et al., 2018).

"Continuous industrial innovation" enabled the European Commission to officially designate 2021 as the beginning of the "Industry 5.0" era (European Commission, 2021). Industry 5.0 complements and extends Industry 4.0. It emphasizes that there will be other determining factors for the location of the future's industries besides economic or technological ones. These factors also have dimensions of environmental, social, and fundamental rights. One should not construe Industry 5.0 as a chronological continuation or alternative to the existing Industry 4.0 paradigm. This concept is the outcome of a forward-looking effort to help frame how industries and emerging societal trends and needs can coexist. Along with the industrial world, the agricultural sector has also started the Agriculture 5.0 era by demonstrating a more holistic perspective as of this period.

This new era, where the concepts of artificial intelligence, big data, the internet of things, virtual and augmented reality, quantum computing, blockchain, and robotics are integrated with autonomous systems, makes it possible to miniaturize inexpensive micro-electronic components through modern manufacturing technologies and to design unmanned robotic systems for automatic control of agricultural resources and mechanisms. Cloud storage systems and high-throughput communication technologies allow for real-time agricultural management decisions. Artificial intelligence systems aid in recognizing reality and controlling drones, tractors, cars, and combine harvesters (Baryshnikova et al., 2022).

The Agriculture 5.0 period also focuses on interdisciplinary applied research and synthesis studies while addressing the main theoretical and methodological approaches to the content, factors, conditions, and methods of the modern technological stage of agricultural development, as well as their impact on food production, food security, and agricultural systems. In this period, the need to use these understandings in culturally, economically, and politically appropriate ways has also arisen to ensure that food is efficiently produced in a more economical and ecological way and distributed in compliance with climate-smart agriculture strategies. Saiz-Rubio & Rovira-Más (2020) argued that the transition from smart farming to Agriculture 5.0 in food production will be accompanied by an even greater increase in productivity and sustainability, as well as the dominance of the ecological aspect of agriculture. Thus, until today, the technological transformation of agriculture has been realized.

2. ROBOTICS AND ARTIFICIAL INTELLIGENCE IN AGRICULTURE 5.0

The Agriculture 5.0 concept represents an era where farms follow the principles of precision agriculture and equipment involving unmanned operations, and autonomous decision support systems are used. Therefore, Agriculture 5.0 uses robots and some forms of artificial intelligence (AI) (Zambon et al., 2019). Farms have needed numerous workers, mostly seasonal, to harvest crops and keep the land productive. Yet, society has moved away from being an agrarian society, with large numbers of people living on farms, to people now living in cities. Consequently, farms have faced the problem of labor shortages. One solution to help with this labor shortage has been identified as agricultural robots integrating AI features.

Initiatives employing robotics and machine learning to solve problems in agriculture started to acquire momentum in 2014, in parallel with the growing interest in AI (Varadharajan, 2017). As the FAO indicated, feeding people and caring for the environment while sustainably increasing production will become even more substantial in the upcoming years. Advanced sensing technologies in agriculture have the quality to help tackle this challenge. Murugesan et al. (2019) noted that the utilization of machine learning and deep learning, along with the employment of AI and robotics, will be capable of predicting yields with a 75% probability and detecting crop diseases.

The agricultural sector is one of the novel machine learning areas where it promises to have a significant impact in the years to come. Many smart agriculture tasks accumulate enormous amounts of data, coming from diverse sources and requiring processing to extract useful information. Therefore, machine learning-based systems are considered a viable solution thanks to their ability to process large numbers of inputs and perform non-linear tasks. In addition, deep learning has recently been used in various research studies, offering modern techniques in image processing and data analysis, indicating enormous potential with promising results. Deep learning is an extension of classical machine learning and takes a more sophisticated approach to predictive models. Thus, it transforms input datasets using various functions. These features result in greater learning capabilities, and thus, higher performance and precision.

Artificial intelligence can provide a computer with the cognitive ability to detect diseases that are likely to occur in a variety of foods, such as fruits and vegetables, and end up causing crop loss. It can also estimate the maturity and harvesting time of these fruits and vegetables. There are multiple ways to estimate ripeness with respect to size, shape, texture, or color. Many of these features can be captured by image or video, and decision-making becomes possible by applying deep learning and AI. After the decision-making phase, fruit or vegetables can be picked through a robotic arm. Mutha et al. (2021) used deep learning to detect tomato-specific maturity in their study. They created a customized image dataset and applied partially automated robotics, employing convolutional neural networks in combination with an object detection model to detect the ripeness of tomatoes and precisely pinpoint their location.

3. INTERNET OF THINGS (IOT) IN AGRICULTURE 5.0

Application of technology to the agricultural sector equips agricultural vehicles and fields with sensors, thus ensuring agricultural vehicles' interconnectedness. Sensors facilitate measuring the humidity, vegetation, and weather, discerning plant species via remote sensing, monitoring stress conditions, drought, soil, and plant conditions, and collecting and analyzing data. Images received from satellites are processed and combined with data transmitted by sensors. Cloud-connected unmanned aerial vehicles can ensure the monitoring of all agricultural terrains, while smart devices can track the retrieved data. Ultimately, the Internet of Things (IoT), a system in which the machines are interconnected, forms the basis of technological applications used in the agricultural sector, and accordingly, big data analysis and smart algorithms can boost productivity and quality. The IoT envisages the utilization of sensors and other devices to transform every farming-related item and action into data. In fact, IoT technologies are one of the reasons why agriculture can yield such a massive quantity of high-value information, and the breakthroughs in these technologies strongly influence the agricultural industry. While in Agriculture 4.0, some of the IoT-based sensors are usually managed using wireless sensor networks (WSNs) through devices, in Agriculture 5.0, this is enhanced through new computing combinations. The combination of Cloud-Fog-Edge Sciences and IoT can be given as an example of this. These new computing combinations make agriculture easier for farmers and agricultural stakeholders by using complementary technologies based on cloud and big data computing to process the vast quantities of data generated by an increasing number of management systems. For instance, the data collected by all devices can include sensitive information and needs to be processed quickly and locally. Here, fog computing benefits local processing and analysis without sending it to the cloud. Edge computing mainly contributes to agricultural applications such as pest identification, safety and traceability of agricultural products, and unmanned agricultural machinery (Zhang et al., 2020). Moreover, edge computing enables the transition to 5G by bringing cloud capabilities closer to end users (Hassan et al., 2019; Markasis et al. 2017; Xu et al., 2017).

Combining the IoT with other computing methods, such as edge-cloud-fog computing, is crucial for data management to achieve maximum benefit in agriculture. If smart farms rely solely on the cloud to analyze and generate results, this may not be a workable solution for real-time data. In this case, a combination of IoT and Edge-Cloud or Fog-Cloud will yield healthier results. In their review, Kalyani & Collier (2021) indicated that most of the recent research on smart agriculture applications focused on IoT and cloud-based systems, while combinations of computing paradigms such as Cloud-Fog-End have just recently begun to be increasingly applied.

4. BIG DATA MANAGEMENT IN AGRICULTURE 5.0

The data concept, coming into our lives with smart agriculture, has introduced us to new concepts over time, along with the various advantages it brings. As smart agriculture technologies became widespread and data concentration increased, the “big data” concept emerged. Besides the large-scale deployment of sensors measuring various properties such as soil moisture, pH, salinity, air humidity, temperature, and evaporation, smart agriculture comprises an enormous amount of heterogeneous data, including images that are retrieved from unmanned aerial vehicles and satellites and produced for agricultural analysis, data from weather stations, and additional sources, all of which in most cases need elaboration, transfer, and recording in real-time via wireless networks. This phenomenon is referred to as Big Data in the literature. The ever-increasing amount of available data for field management imposes the need to implement some sort of automated process to extract operational information from aggregated data. Big data is analyzed in five dimensions, including the last one identified by Chi et al. (2016). These dimensions are denoted as volume, velocity, variety, veracity, and value.

The volume dimension refers to datasets that are beyond the ability of typical database software tools to capture, store, manage, and analyze information. This definition includes an estimation of how large a dataset should be so that it is deemed big.

Velocity connotes the ability to capture, understand, and interpret events as they occur. In agriculture, this describes real-time applications, such as data processed directly in the field for applying chemicals at variable rates on equipment through variable rate application technologies.

Variety implies different data formats (videos, text, audio) and varying degrees of complexity. This dimension pops up in agriculture when different data sources are employed to operate on complex scenarios, such as imaging technologies and soil or air sensors.

It has been confirmed that big data is increasingly applied in the agricultural sector (Anonymous, 2022). Kamilaris et al. (2017) presented 34 studies using big data in agricultural applications. In line with this trend, the International Consortium of Agricultural Research Centers (CGIAR, Montpellier, France) created a platform for Big Data in Agriculture to utilize big data approaches to solve agricultural development problems faster, better, and on a larger scale (CIAT & IFPRI, 2016).

Although Big Data in agriculture is still at an early stage, it has the potential to emerge in various applications. Weather forecasting is an important application where local or global weather data must be processed to support decision-making systems that will assist farmers. The amount of data generated for weather forecasting is huge and requires analysis and processing in real time, adding additional complexity. Big Data is also present in crop production forecasting employed by global monitoring systems to provide data analysis tools for product development status monitoring and production evaluation (Becker-Reshef et al., 2010). Weed separation is another area where substantial amounts of data must be analyzed, processed, and used by multiple machines in the field. In addition, newly collected data should be used to improve existing algorithms for weed control. Storing and querying this amount of data poses significant challenges. Detailed knowledge of planted areas based on accurate remote sensing technologies is a key parameter for land management (Barrett et al., 2014). This will culminate in increased productivity. All these applications generate input parameters for decision-making systems to assist farmers in their decisions (Moysiadis, 2021).

5. BLOCKCHAIN-BASED AGRI-FOOD SUPPLY CHAIN MANAGEMENT

Agricultural production, for the economy of a country, demonstrates integrity with the safety, nutrition, and health of its people. Agricultural practices involve numerous options and sensitivities, such as weather continuing to change from season to season, agricultural products' market prices continuing to fluctuate, soil quality declining, crops not being sustainable, weeds and pests damaging crops, and global climate change. The agricultural production process is a whole, and developments at each stage have the potential to affect the previous or the next step. Therefore, the process must be traceable for analyzing food quality, storage conditions, weather in a given geographical area, soil quality parameters such as pH, salinity and nutrients, marketing and trade management, and the presence of food hazards.

Traceability begins in the cropland and continues throughout the supply chain. Food safety and competition are the primary impetus for implementing food supply chains where traceability from the source to the retailer is a distinctive feature. Traditionally, information and communication technologies (ICTs) used to be employed for tracking data, maintaining information flows, and databases. In cases where clarity is missing in agricultural supply chains, concern about the financial losses involved rises, end-user trust gets affected, and corporate brand values indicate a decline. Accordingly, the requirement to make several fundamental changes to the current supply chain architecture for the establishment of an efficient and trustworthy trading environment has emerged.

Blockchain technology, coming to the forefront alongside Agriculture 5.0, emerges as a new way of powering these databases. This technology grants rights to all network participants, rather than a single server and administrator. Moreover, multiple parties can access and verify new entries to the database (Anonymous, 2022a). There is a broad consensus that blockchain can increase transparency in agri-food supply chains. In agriculture, blockchain technologies can make it easier to trace any contamination or other issues back to the source in a call-back. Consumers now demand safe, sustainable, and fair food production processes, so businesses are using blockchains and the internet of things to meet these needs.

Blockchain is defined as a chain of blocks containing information. Each block records all current transactions and, upon completion, stores them on blockchain as a permanent database. In a blockchain, a block is a secure server, both time- and date-stamped, and at the same time, a secure cross-party database. This technology is considered a decentralized, distributed system that records the origin of a digital asset. Blockchain technology brings together agriculture and various sectors and has the quality to make a reform within the new agricultural revolution (Singh & Singh, 2020). Bermeo-Almeida et al. (2018) argued that blockchain would help solve most of the security and reliability-related IoT problems.

Today's agricultural supply chains are evolving from sovereign and autonomous local stakeholders to a globally interconnected system of multiple participants connected through complex interactions and influencing the production, processing, transportation, and distribution of food to end consumers (Bhat et al., 2021). Therefore, the strategic process of managing the entire journey of agricultural products from the initial targeting stage to development, service, and delivery to the end user becomes traceable.

6. CLIMATE-SMART AGRICULTURE 5.0

Despite the positive developments experienced throughout the history of agriculture, there are still problems of critical importance. Today, food and farming account for approximately 30% of global greenhouse gas emissions. The agricultural industry also accounts for the highest amount of freshwater use. The way we feed ourselves is one of the primary drivers of biodiversity loss. Existing food and agriculture systems affect planetary health. Humanity has reached a point where food systems must reconcile the need to produce enough healthy and affordable food with the imperative that we do not disrupt the ecosystems we depend on for life. By 2100, around 10 billion people will need carbon-free food systems that are resilient to extreme weather conditions and have the potential to provide positive socio-economic benefits to all participants in the food chain. Ensuring food safety in these circumstances requires innovative approaches.

The agriculture sector has undergone a transformation that will enable it to exceed productivity and profitability in response to changing land conditions because of the climate crisis, driven by promising recent technologies, by using precision farming methods along with Agriculture 4.0, and the process is in progress. The digital agriculture revolution has inspired positive developments, especially in terms of access to markets, a focus on low-cost vehicles, and promoting food security in some parts of the world. Although the Agriculture 5.0 concept implies that farms are following the smart agriculture principles, yet taking this process further and using the equipment including autonomous decision support systems and unmanned operations, a purely technology-based view of Agriculture 5.0 and a range of other equally important strategies for addressing the social and political aspects of food and farming systems need to be concomitantly presented, as with Agriculture 4.0. It is appropriate to consider Agriculture 5.0 with Industry 4.0 and Society 5.0, to develop environmentally sound technologies, to create sustainable smart cities, and to deem industrial development as the standard of human well-being (Berawi, 2019).

Agriculture 5.0 aims to create a user-friendly, real-time, and cost-effective smart system that will help farmers monitor and manage their farms with less power consumption and less greenhouse gas emission in all its innovative approaches. Ali et al. (2018) proposed the “G-IoT” approach, characterized as the “green internet of things” that will function as a decision support system. They defined that concept as “energy-efficient procedures (hardware or software) adopted to ensure that the IoT facilitates greenhouse effect reduction of existing applications and services or that the greenhouse effect of the IoT itself is mitigated."

The technological breakthroughs inspired by green energy source use will lead to change and greater efficiency for the agricultural industry. As the farmers have a long-term source of income by using green energy sources such as wind, solar, and biomass, they will manage to reduce their carbon footprint and make their businesses more efficient. One of the most widely used green energy sources for a smart farm is biomass energy. Biomass energy can be produced out of plants and organic wastes (De Oliveria et al., 2022). Although maize is the most widely used energy crop at present, endemic prairie grasses or fast-growing trees such as poplar are likely to become more popular as energy sources in the future (Dhunny et al., 2019). These perennial plants are lower-cost and more sustainable for energy production. The wastes from these plants can be converted into energy, which can be used in several ways, by smart farmers to heat their buildings, power their smart devices, or use as transportation fuel for their smart vehicles (Fontaras et al., 2012; Pisano et al., 2021; Ragazou et al., 2022).

7. SUSTAINABILITY APPROACH IN AGRICULTURE 5.0

The growing world population, climate change, and sustainable development goals require the agricultural industry to be efficient and sustainable, thus ensuring food security. It is estimated that approximately 690 million people suffered from hunger in 2015. Especially with the COVID-19 pandemic, this situation has been exacerbated by the loss of income due to the impact on food supply systems and increasing food prices. In this context, more attention has been paid to the efficient integration of smart farming technologies into agriculture to address the sustainable development goals, especially the second goal of “zero hunger." At the present time, for a highly productive and sustainable agriculture, smart farming technology applications are indispensable. Therefore, integrated with the fifth industrial revolution, a particular importance on remote sensing and precision agriculture technologies, which are the primary platforms of this technology, has been attached in the fifth agricultural revolution. For instance, in this period, remote sensing technologies aimed to increase their effectiveness in achieving sustainable agriculture by directing existing research toward the estimation of plant physiological characteristics rather than structural parameters (Martos et al., 2021). Likewise, the 13th goal, “climate action,” and the 7th goal, “affordable and clean energy," which are among the sustainable development goals, conform highly with the understanding of “climate-smart agriculture 5.0”. Especially with the Green Deal, the obligation of most producers and companies to switch to green technologies to ensure both social and economic sustainability is in line with the understanding adopted by Agriculture 5.0. Through the smart agricultural technologies that have developed with the Agriculture 5.0 era, they will both minimize the use of inputs in production and manage the process in a greener cycle at every stage of production by shifting to renewable energy sources and obtaining clean energy. Again, within the agriculture process ongoing with Agriculture 5.0 and acquiring more and more popularity, the employment of blockchain technology will ensure further alignment with the target of “responsible production and consumption," which is the 12th of the sustainable development goals, owing to the product tracking systems that increase both forward and backward traceability of the process from the production stage to the end user, as the increased food safety will allow conformity with the 3rd goal of “health and a quality life”.

8. CONCLUSION

Undoubtedly, we are in a period of enormous transformations in the production, distribution, and consumption of agricultural products. Globally, IoT and AI technologies are helping solve pressing issues across the agriculture value chain. The concept of smart agriculture is essential to address the current challenges relevant to productivity, climate change, food security, and sustainability in agriculture. However, it is not enough to increase the production process of agricultural products alone. It is also important to ensure and obtain high nutritional value as well as the required safety. Thus, to address all the above issues, building agro-ecosystems based on emerging technologies is gaining importance.

In addition, this chapter also depicts that the transition from smart agriculture to the new "digital age" of Agriculture 5.0 is based on the principle of being driven by land management compatible with the use of green energy sources, the modern model in agriculture. Agriculture 5.0, with green energy sources and smart tools, is moving in the right direction to move from a typical smart farm to an energy-smart farm. Thus, Unmanned Aerial Vehicles (UAVs), sensors, smart tractors, and much more can be achieved through the utilization of various smart devices based on emerging information communication technologies, artificial intelligence, machine&deep learning, big data analytics, and blockchain technologies at low cost and in an environmentally friendly way. The Agriculture 5.0 era is a crucial technological component that will enable agriculture to increase its productivity and efficiency in all aspects while providing great opportunities to mitigate and prevent new crises caused by climate change, rapid population growth, and environmental degradation.

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CHAPTER 2

PRODUCION OF PLANT BIOACTIVE COMPOUNDS WITH PLANT ORGAN, TISSUE AND CELL CULTURE TECHNIQUES

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INTRODUCTION

Plant bioactive components are important for the food, pharmaceutical and cosmetic industries. Plant bioactive compounds can be primary metabolites such as glycoses, amino acids, lipids and vitamins or secondary metabolites such as alkaloids, resins, and essential oils (Begum et al., 2022). While primary metabolites are essential for plants survival, secondary metabolites are produced for such as their defence, as pheromones and storage waste metabolites (Hussein and El-Anssary, 2018). There are 210 small molecule pharmaceutical raw materials in the WHO's model list of essential medicines, in which 17 compounds are plant originated (Başer, 2012).

Since plants have no movement ability, they have undergone significant changes to adapt, compete or fight with their changing environment. Natural selection made possible for plants to reach the present day with significant variations in the plant kingdom. For instance, plants secrete odour attracts pollinating bees or bitter, although poisonous taste or allelopathy chemicals preserve against to predators and rivals. The bioactive compounds that isolated from plants are used in pharmaceuticals. The growing market of medicinal and aromatic plants is expected to reach 5 trillion $ by 2050 (Chandran et al., 2020). The phytochemical compounds that produced by plants are used in the food industry as food supplements, aroma and flavour components, as well as in medicine as pain relievers, tranquilizers, cough suppressants or plant based natural additives in pharmaceutical industry (Yu et al.2021).

Herbal metabolite yield is unstable and production costs are high under natural conditions (Chandran, 2020). Phytochemical yields, which are very low in field conditions, are maximized and phytometabolites are obtained at stable levels by suspension culture method. And bioactive chemical production yield can be standardized by tissue culture methods, regardless of locational and seasonal conditions and chemical contamination risk is reduced for bioactive component. The highest potential production yield is optimized through in vitro tissue culture methods. Bioactive product production is preferred by the pharmaceutical industry as a reliable raw material source. Efficiency and quality in production processes are standardized with biotechnological methods. Bioactive metabolite yields are increased into considerable level using organ, tissue and cell culture methods (Wawrosch and Sergey, 2021).

TOTIPOTENCY AND MICROPROPAGATION

Plants can regenerate different organs from their differentiated tissues thanks to their totipotency properties. The feature makes clonal reproduction possible (Feher, 2019). The tissue culture method is an important example of the vegetative production method under sterile laboratory conditions. It is possible to reproduce organs, tissues or cells under sterile conditions. The artificial medium contents and controlled environmental conditions provide a uniform multiplication continuously for along year and on different location (Bhatia et al., 2015). Tissue culture methods also provide an uninterrupted production opportunity free from disease, chemical contamination, pests and virus infections. Bioactive production is more suitable with tissue culture methods compared to harvesting and processing plants in hectares under natural conditions (Espinosa-Leal et al., 2018).

Plants used for tissue culture techniques proliferate under conditions of artificial lighting and air conditioning. The method provides geometrical reproduction ratio, aseptic and clonal plant production throughout the year since *in vitro* plant production conditions independent from environment and seasonal variation. Tissue culture method is a method of commercial reproduction of plants with high economic value, as well as it is an important method for breeding purpose, ex-situ protection, medical, pharmaceutical, cosmetic and food additive bioactive substances can be reproduced at intense rates. Bioactive products can be produced by following bioreaction processes with cell suspension cultures. The bioactive products can be many plant cells as well as medicinal raw materials, cosmetic ingredients, pharmaceuticals and plant growth regulators (Motolinía-Alcántara et al., 2021).

ESTABLISHMENT OF CULTURE CONDITIONS

A plant tissue culture laboratory should consist of at least 4 four separate rooms (Mather and Roberts, 2002). First is a preparation room where the nutrient medium is prepared, pre-sterilized and the nutrient medium vessels are cleaned, the second is a transfer room where the culture applications take place under sterile cabinets, the third is a climate room where the plants grow on shelves under artificial lights and the forth is a acclimatization room or greenhouse where plantlets are acclimatized to the outside environment (Figure 1). Bioreactor and devices for extraction and chromatography will be beneficial when bioreaction process are aimed (Mineo, 1990).

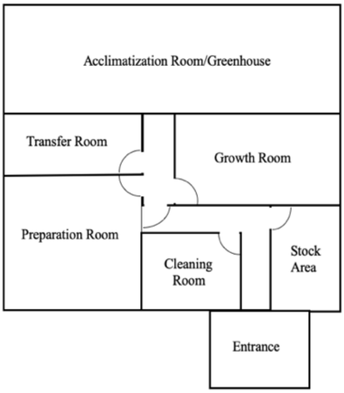

Figure 1: Basic Tissue Culture Laboratory Plan

Tissue culture laboratory conditions must be extremely sterile. Plants must be protected against microbial contamination risks. Sodium hypochlorite, calcium hypochlorite, ethanol, mercury chloride, silver nitrate, ozone, biocides and fungicides are the most commonly chemicals for plant surface sterilization. The plants taken into the culture medium are washed three times with liquid soap for surface cleaning against macro contaminants. Ethanol at 70% concentration for 30 seconds and 20% sodium hypochlorite for 20 minutes is applied. The sterilant concentration and duration can be increased in woody parts, seeds and underground organs, but it can be decreased for young parts, shoots and sensitive parts of plants. Plant parts are rinsed with sterile distilled water at least three times under sterile cabinet at the end of period and the plant parts are placed appropriate nutrient media which is autoclave sterilized.

The media protocols used for plant tissue culture methods were composed in accordance with the plant morphological conditions and their optimum nutrients. Murashige and Skoog (1962) generated a media for tobacco that is still used for many plant species. But many plants need some specific protocols and they have been called after their inventor's names. For instance, Chee and Pool (1987) for grape and Quoirin and Lepoivre (1977) is for Prunus developed media by removing chlorine and iodine from the environment. Anderson (1980) is for *Rhododendron* and berry groups. Chu (N6) media were developed for monocotyledon anther cultures. These groups are sweet potato (Chee, 1992), sugar beet (De Greef and Jacobs, 1979), walnut (Drive and Kuniyuki, 1984), soybean (Gamborg et., al., 1968), orchid (Morel, 1965), carrot or Pinus (Litvay, 1985), for woody plants (Lloyd and Mc Cown, 1980), for olives (Rugini, 1993). Uptake forms of nutrients are important while establishing a protocol for a plant specy. For instance, high quantity of calcium and sulphur forms calcium sulphate and it is not soluble in water. Antagonist and synergist effects and plant growth regulator derivatives are also significant for media contents.

Plant media should occur in macro and micro nutrients, vitamins, amino acids, sugar, hormones, organic compounds, plant protection products and solidifier. The main task of the nutrient medium is to ensure healthy development of plantlets. Reproduction is quite fast related with incremental explant rate geometrically. When each explant produces 4 new shoots in the meristem culture method, approximately 1 million clones are obtained at the end of a year with 30-40 days of subculture processes. When the reproduction number of new shoots to be obtained is five, the approximate number of clones will be 10 million theoretically at the end of the year.

Ambient temperature, relative humidity in the container, light frequency, photoperiod is effective on plant growth. Also, artificial lighting photoperiod and density is significant where plants placed. For example, heliophyte plants prefer intense lighting intensity, while xerophyte plants prefer low intensity.

ORGAN CULTURE METHOD

Purpose for producing bioactive compounds can be variable in term of organs in which they accumulate of plant raw material in plants so different organs are preferred. For instance, Astraglocide IV accumulates in *Astragalus membranaceus* plants roots, vinblastine and catharanthine accumulate in the *Catharanthus roseus* leaves, and diosgenin in the tubers of *Diocorea* spp. Plant growth regulators manipulate regenerations. While cytokinin and gibberellic acids are preferred for root development and shoot regeneration, auxin-derived growth regulators are preferred for root regeneration. Chlorocholine chloride (CCC), paclobutrazol (PBZ) and sugar concentration increases tuber regeneration (Zheng et al., 2012). *Rhizobium rhizogenes* bacteria inoculation is also possible for root regeneration, which causes hairy root formation. Hairy root culture is used for *Catharanthus* spp phytochemicals which are alkaloids, atropine, and hyoscyamine (Shakeran et al., 2017).

Plants can grow in liquid solutions that air-saturated in organ culture media. The method is called Temporary immersion system (TIS). It is an enable method for proper plant growth because it is ensured as regular fresh air flow is provided to the nutrient medium. The most important point should be considered as contamination risk in the TIS. The air flow should not provide contaminated air flow to the sterile plants are located (Georgiev et. Al., 2014). TIS working principle is given in figure 2.

Two sterile containers, which are enabled to transfer liquid media to each other with a capillary hose, the upper one contains explants and the nutrient media solution in the lower one. There is an air compressor connected to the lower container. Air supply is provided by an air pump and fresh air is passing through hepafilter. There are two situations in the system. In the first case the air compressor works, in the second case the air flow is cut off. In the first case when the air compressor provides clean fresh air to the lower container, the media inside the container is transported through a capillary tube to the upper container by the effect of positive air pressure. Plant parts in the upper container are treated with a nutrient solution it is saturated with fresh air. After a while, the compressor is turned off. In this case, with the effect of gravity, the nutrient solution flows into the container below, while the plants are kept in the upper container with the help of a filter. While air pump works the nutrient solution flows into the container (Figure 2). TIS used commonly for banana, potato micro tuberous (Bello-Bello et.al., 2019, Jimenez et.al., 1999).

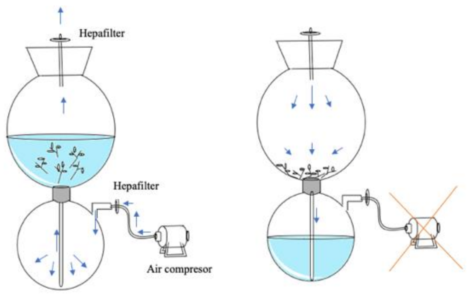

Figure 2: Temporary Immersion System Working Principle

TISSUE CULTURE METHOD

Meristem culture is the most commonly used tissue culture method for clonal micropropagation, so it is called as tissue culture. Tissue culture methods are resistant to somaclonal variations, mostly used in clonal multiplication commercially. Another tissue is secretion is very important in term of tissue culture. Glandular trichomes (hairy) sacs on the stem and leaf are very useful for medical purpose. The methods in which the volatile components in the secretory tissues are reproduced by tissue culture methods can be exemplified. Fragrance components aromatic components can be reproduced with tissue culture method. For example, plumbagin 1,4-naphthoquinones and 7-methyljuglone are obtained from the glandular hair of the *Drosera capensis* (Budzianowski, 2000).

CELL CULTURE METHODS

Cell cultures are created from individual cells from which callus or cell walls have been removed. Cell cultures can be used in suspension media as well as in solid and semi-solid media. Since cells are independent in cell cultures, their signal transduction is also cell-independent and unaffected by gene expression from neighbour cells. Cells can be regenerated into organs producing bioactive substances with the help of growth regulators, and valuable bioactive molecule yields can be increased with heavy metals, elicitors or measured biochemicals. The tissues from which the starting cells are supplied are very valuable in terms of active substance yield. For example, starting cells from different organs of *Morinda citrifolia* were differed in term of anthraquinones yield (Deshmukh et al., 2011).

Plant cells can act independently like bacterial cells in the cell suspension culture medium. Cells are kept mobile in culture vessel with agitation or fresh air supply, otherwise plant cells will colonize. While the process is similar to microorganism cultures, some basic differences are observed in the nutrient media as plants are heterotrophic organisms. On the other hand, processes can be carried out under fully controlled environment conditions such as a bioreactor.

It is possible to reproduce autonomously the living cells in the suspension culture medium with the help of a bioreactor. A bioreactor is a computer-controlled device that controls the nutrient solution feed, pH, EC, clean air intake, and bioreaction processes in the nutrient medium (Figure 3). The targeted concentration of bioactive substances in the suspension is also important (Espinosa-Leal et al., 2018). Amount of the maximum growth and duration of the cells vary depending on the consumption rate of the nutrient medium (substrate) in the bioreactor environment (Figure 4). In addition, phenolic or toxic compounds secreted by plant cells into the environment also determine the length of the production period. Cell yield stabilization is effective in determining the bioreactor type (Motolinía-Alcántara et al., 2021).

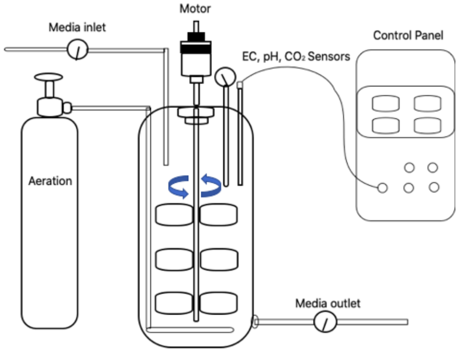

Figure 3: Bioreactor Working Principle

Bioreactors are divided into three types as continuous, batch and semi-batch. In continuous reactors, feeding and harvesting continue uninterrupted during the bioreaction process. In batch bioreactors, no feeding or harvesting takes place during the reaction process, end of process the tank is cleaned, feeding and cell cultivation takes place from the beginning. In semi-batch bioreactors, before reaching the decline phase, enough cells are harvested, and new nutrient medium is added to the medium (Figure 4).

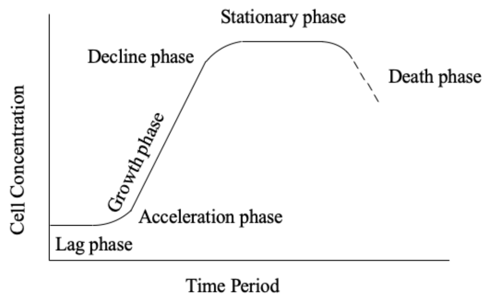

Figure 4: Cell concentration changes related with time period in a suspension culture

PATHWAYS OF BIOACTIVE COMPOUNDS

Plant anabolism activities, which start with the conversion of solar energy into chemical bond energy, produce glucoses as a result of photosynthesis reactions. These basic functions take place in chlorophyll cells, which contain the green pigment. Under the light, the carbon obtained from the carbon dioxide molecule, and the hydrogen and oxygen obtained from the water molecule are used in the production of glucose. Photosynthesis constitutes an important and fundamental step in our food chain. Glucose is converted to pyruvate via the glycolysis pathway, and the conversion of pyruvate to Acetyl Co-A molecule creates the raw material for the citric acid cycle. The main cycles in which primary and secondary metabolites are produced. Primary and secondary metabolites such as nucleic acids, amino acids, vitamins, and fatty acids occur with these two important cycles, glycolysis and citric acid cycle. For example, phosphoenolpyruvate is an input to the shikimic acid cycle, while flavonoids are produced at the end of this cycle. Acetyl CoA, the end product of the glycolysis pathway, is the raw material for the krebs cycle, as well as the for the reactions that will form phenolic compounds with the malonic acid cycle, and terpenes and alkaloids with the mevalonic pathway. By using TCA or Krebs cycles, the concentration ratios of some plant amino acids and nucleic acids in the plant cell can be increased naturally (Figure 5)(Zhang and Fernie, 2018; Alabduladhem and Bordoni, 2021).

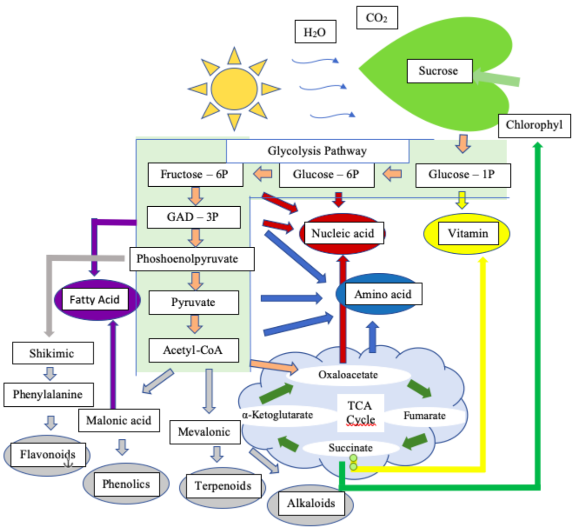

Figure 5: Metabolic pathways for bioactive compounds

APPLICATIONS FOR BIOACTIVE SUBSTANCES IN THE CELL

The concentration of nutrients in the plant nutrient medium, light, pH, temperature, hormones, elicitors, precursors, agitation speed, osmotic pressure, abiotic or biotic stresses, organic or inorganic compounds and some microorganisms can increase the production of bioactive compounds (Sökmen and Gürel, 2002).

Elicitor is a plant defence secretion is produced by pathogen attack. The elicitors can be used for bioactive compound production in suspension culture systems. Methyl jasmonate (MeJA), NaCl, $AgNO_3$ are the most common elicitors used in tissue culture media (Cetin and Baydar, 2014). The biochemicals like salicylic acid, cinnamic acid, coumaric acid, caffeic acid, and ferulic acid are called as precursor (Faramayuda, 2022). Precursors are raw materials for a biochemical reaction in a metabolic pathway. Also stress factors such as aluminium has a possitive effect on callus development for 1,3-β-D-glucans production. Cadmium sulphate (CdSO4) is also another stress factor used in suspension cultures (Bhuja et al., 2004).

Clinical tests and pharmacologic studies are extremely necessary for a plant bioactive material to use for medicinal and cosmetic purposes. Bioactive samples should be superior to placebo statistically. Herbal medicines need to be standardized in terms of botanical, physical, chemical and biological aspects. Botanic standards: such as colour, odour, taste, texture, fracture, qualitative and quantitative characteristics; chemical standards: chromatographical features, heavy metal, pesticidal residue; physical aspect: moisture content, solubility, ash, spectroscopic and biologic purpose: microbial, pharmacological and toxicological are classified (Shivatare, 2013).

Bioactive compounds can be produced by substrate-product relationship in tissue culture techniques. Along gene transfer, or synthetic biology methods are also used for biotechnological drug production (Liu and Stewart, 2015).

Plant bioactive metabolites accumulate in different plant cells, tissues and organs. Regenerable cells may be differentiate to different tissues and organs such as meristem, roots, shoots, corms, bulb, meristem, and secretory tissues. The accumulation rates in the cell can be controlled when *in vitro* conditions are controlled. Suspension cultures can augment bioactive components on a cellular basis. The ability to produce tissue-based biochemicals with these methods forms the basis for the intensive reproduction of herbal active substances used for pharmacological purposes (Yancheva and Kondakova, 2016).

When the plant parts or extracted compounds are used for pharmaceutical purpose. The bioactive materials must hold the standard and reliable purity. The most important advantage of a bioprocess application is standardization of a bioactive substance industrially. Validation processes also refers bioactive material accumulated in the plant cells reaches the highest concentration, the information about the purification of the bioactive materials and the most ideal sample preparation methods for the chromatographic methods. Validation has three steps are designing, qualification and process verification. (Sumeet and Gurpreet, 2013).

Industrial downstream process applications should be applied the in upstream process at the initial stage. The upstream process is a step that also examines the validation processes of the bioactive substance. The bioactive substance amount in plant cells changes with altitudinal, seasonal, climatic conditions and soil structure in the field. Upstream processes occur in 4 steps. First stage is pre-sterilization of plants, second is transfer to nutrient media and callus development, third is increasing the dilution rate of the active substance with the help of some applications in the bioreactor, extraction of the active substance and determining the amount of active substance by extraction and analysis is the last stage (Figure 7).

Downstream processes are used for the industrial production of bioactive substances with satisfactory market economic value for bioactive compounds (Tripathi and Shrivastava, 2019). The downstream stages are created with the analytical information obtained by the upstream process. Downstream applications have higher volume media, equipment and end products than upstream stages. The downstream process starts with the plant cells obtained in the upstream. The ratio of bioactive substance concentration is increased in the upstream process (Gronemeyer et al., 2014). In the process, it is very important to take high sterilization precaution against contamination risks. When the cell concentration reaches the decline phase, cells are harvested. Bioactive substances separation step is started after the harvest. The biochemical product must be exact purification. The bioactive compounds in the cells are extracted, controlled in term of quality and packaged. The purification step of bioactive substances is very important in terms of the industrial evaluability of the product quality (Figure 7) (Kocabaş, 2017).

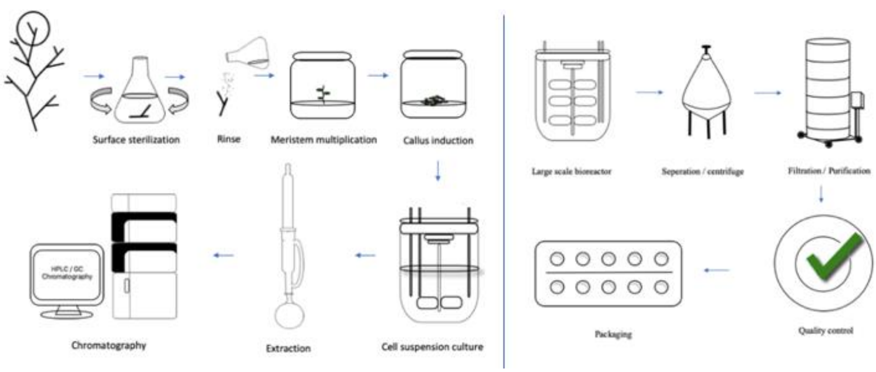

Figure 7: Upstream and downstream process for bioactive compounds production

INDUSTRIAL SCALE BIOACTIVE COMPOUNDS PRODUCTION

Bioactive compounds have an important market value as human nutrition, food additives, pharmaceutical cosmetics, etc. The number of active ingredients is as high as number in the plant kingdom. Since it is easier to reach plant bioactivated products in nature than microorganism products, it has not been much used until recent. However, an important market has also emerged for high-quality bioactive compounds, since reaching high-concentration effective substances requires high technology. As seen in Table 1, producers of bioactive compounds operate in different parts of the world.

Table 1: Some of Bioactive Compounds and Manufacturers (Compiled from Ochoa-Villarreal M., 2016)

Plant	**Bioactive Compounds**	**Manifacturer**
Coleus blumei	Rosamarinic acid	A Nattermann & Cie. Gmbh,Germany
Digitalis lanata	Digoxin	Boehringer Germany
Echinacea purpurea	Echinacea polysaccharides	Diversa, Ahrensburg, Germany
Nicotiana spp	Vaccine against Newcastle virus (NDV)	Dow Agrosciences, LLC, USA
Physcomitrella patens	Human proteins	Greenovation Biotech BMBH, Germany
Geraminea spp.	Geraniol	Kanebo Japan
Carthamus tinctorius	Carthamin	Kibun Foods Japan
Geraminea spp.	Geraniol	Mitsui chemicals., Inc
Catharanthus roseus	Arbtin	Mitsui Chemicals Inc., Tokio Japan
Lithospemum erythrorhizon	Shikonin	Mitsui Chemicals., Inc
Coptis japonica	Berberines	Mitsui Chemicals, Inc
Coleus blumei	Rosmarinik Asit	Natterman Germany

Table 1: Continued

Euphorbio milii	Anthocyanins	Nippon Paint Co. Ltd, Japan
Beta vulgaris	Betacyanin	Nippon Shinyaku Co., Ltd
Podophyllum spp.	Podophyllotoxin	Nippon Oil, Tokio, Japan
Taxus spp	Paclitaxel	Phyton Biotech., Inc Germany
Daucus carota	Human Glucocerebrosidase (GCD) enzyme	Protalix BioTherapeutics, Israel
Atropa belladonna	Camptothechin Atropine,	Rootec, Witterswil, Switzerland
Nicotiana glauca	Nicotine, Anabasine	Rootec, Witterswil, Switzerland
Panax gingsen	Gingsenosides	Rootec, Witterswil, Switzerland
Duboisia spp.	Scopolamine	Sumitomo Chemical Co., Ltd, Japan

These countries are specifically industrialized. Japan, USA, Germany, Switzerland is among the countries that produce bioactive compounds with reliable content. The plants listed in Table 1 are generally plants that are raw materials in traditional medicine. It is known that almost all of these plants are produced by suspension culture and hairy root bioreaction method (Akın, 2020).

MOLECULAR FARMING AND BIOTECHNOLOGIC APPLICATIONS

Biochemical processes are controlled by genes. It is possible to transfer a targeted nucleotide to other living organism and the biochemical processes will be expressed in the host plant (Singh et.al., 2021). Recombinant protein, vaccine, enzyme, human growth hormone genes can be transfer to the host plants. The method is called molecular farming. Suspension culture and hairy root methods are used in corn and tobacco species with molecular farming mostly (Horn et al., 2004). Tobacco, rice and soybean is the other plants are used to aim of molecular farming (Table 2). Crispr Cas9 is an enzyme can be used for cutting off a DNA location and replace with another for produce a specific pharmaceutical (Liu et al., 2017). Plants are suitable organisms for gene applications because applications with plants do not require ethical report and they can live very basic conditions.

The organism has smallest genome are aimed for pharmaceutical application. It means no extraction cost and high purification standards for bioactive substances. It is the discipline known as synthetic biology. It is possible that many different gene applications will be mentioned in the near future.

Table 2: Some molecular farming studies and the products (Compiled from Horn et.al., 2004)

Compounds	**Host Plant**	**References**
Avidin	Corn	Kusnadi et al., 1998
B-Glucuronidase	Corn	Evangelista et al., 1998
Trypsin	Maize seeds	Woodard et al., 2003
Aprotinin	Corn	Zhong et al., 1999; Delaney et al., 2002
Human Gastric Lipase	Tobacco	Gruber et al., 2001
Human Lactoferrin	Rice and Corn	Samyn-Petit et al., 2001 Nandi et al. 2002; Legrand et al., 2003
Edible Vaccine Hepatitis B, Transmissible Gastroenteritis Coronavirus (TGEV)	Corn	Lamphear et al., 2002
Human Immunodeficiency Virus (HIV)	Corn	Horn et al., 2004
LT-B Vaccine Candidate	Corn	Tacket et al., 2003
Monoclonal Antibodies Iga	Tobacco	Frigerio et al., 2000
Genital Herpes	Soybean	Zeitlin et al., 1998

CONCLUSION

Plant kingdom has vast number of species, so they show many bioactive component variations can be used for industrial purpose. The bioactive products are used as pharmaceutical products and they have an important market value. These products are used for human and veterinary needs such as cosmetic, medicine, nutrient, food additive, etc. For this reason, reliability in the production method is very important for these products. It is very important to produce and purify the bioactive molecular produced in plant cells at high concentrations.

In this review, the production of plant bioactive products by organ, tissue and cell culture methods is explained. It is possible to apply the upstream and downstream processes for the production of bioactive components by plant tissue culture methods on an industrial scale. Traditional herbal products are realized in the production of reliable technological bioactive components, which take place in industrialized countries. It is quite possible for these products to be included in the production processes in developing countries as well.

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CHAPTER 3

USE OF BIOREACTORS IN PLANT BIOTECHNOLOGY

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INTRODUCTION

When the course of agricultural production in the whole world is examined throughout history, two important periods that can be described as revolutions stand out. The first of these periods is the "green revolution" that emerged with the acquisition of superior varieties, the application of commercial fertilizers, and advanced agronomic techniques, and the other is the "gene revolution", which has become increasingly influential, especially in the last 40 years and has emerged with the application of plant biotechnology.

It is assumed that the world population will reach around 11 billion in 2100 with a normal growth rate, in the case that the available resources in the world at the end of the 21st century should feed a population close to twice the current one. Contrary to this, the gradual shrinking of agricultural areas has not been considered as a problem for many countries. The reason for this contrasting situation is that the recent increases in yield have more than closed the gap caused by the contraction in agricultural areas. However, the increase in yield has fluctuated with various factors over the years. Preventing this fluctuation and increasing productivity is a necessity in the face of increasing population and consumption. The need for superior technologies is becoming more and more important, as there is no clear answer to whether the increase in yield can compensate for the loss of yield caused by the shrinkage in agricultural areas.

Plant biotechnology, which offers many possibilities for the manipulation of biological systems, has led to the emergence of promising studies in plant-related sciences. Plant biotechnology is aimed to improve plants at the molecular level by using various tissue cultures and genetic engineering techniques. For example; many plants from herbaceous and woody taxa can be propagated intensively and clonally, or the active substances produced by many cultivated plant species can be produced under laboratory conditions. In addition, genes of agricultural importance can be easily transferred to cultivated plants by isolating them from different organisms.

There is an absolute need for tissue culture methods in the improvement of plants with biotechnological methods (Babaoğlu et al. 2002; Chawla, 2002). In other words, *in vitro* tissue culture techniques are the most essential methods that can be used for genetic improvement, selection, and intensive and clonal propagation of plants.

It is known that only about 3000 plant species out of approximately 250 thousand have nutritional value in the whole world flora (Chawla 2002). Two important periods stand out in the studies on the improvement of plants for use in human and animal nutrition. The first of these is the so-called 'Green Revolution', the period when the development of classical plant breeding, commercial fertilizers, and other agronomic techniques was effective. In the Green Revolution, production takes place in the seed-plant-seed cycle. The second period is called the 'Gene Revolution' (Kung, 1993). Understanding the structure of DNA, bacterial genetics, the development of plant tissue culture, and the applicability of these techniques to many plants started the gene revolution period. Compared to the green revolution, there is an induction in terms of reaching the goal of the gene revolution. Each stage of the plant-seed chain has the capacity to reach the goal, be evaluated separately, and make changes, owing to the totipotency feature of plants. The part from these subjects to the cell constitutes the fields of interest of genetic engineering, and of plant tissue culture on the subjects between the cell and the seed. In short, the main elements those makeup plant biotechnologies are genetic engineering and plant tissue culture studies.

Plant tissue culture is made under aseptic conditions, in basal media, from plant parts such as a cell (meristematic cells, suspension or callus cells), tissue (various plant parts = explant), or organ (apical meristem, root) production of products (such as metabolites). Developing new cultivars and creating genetic variability in existing cultivars are among the main purposes of tissue culture. Therefore, plant tissue cultures play an important role in genetic improvement studies. In addition, different methods of tissue culture are routinely applied in the production of species that are in danger of extinction or have some problems in reproduction by natural methods.

The basic system used in both plant tissue culture and genetic improvement studies is plant regeneration. In this system, the properties of cells can be examined in three parts;

1) regeneration from somatic tissues containing organized meristematic cells,
2) regeneration from non-meristematic somatic cells, and
3) regeneration from gametic cells that have undergone meiosis.

Reproduction of plants from shoot tip and lateral meristems is the first type of regeneration. The cells obtained from this regeneration are completely similar to the donor plant. In the second type of regeneration; directly on the cut surfaces of a plant explant, some of the specific somatic cells divide and organize, usually as a result of the action of plant growth regulators (especially auxin and cytokinin), organ, and then the plant (direct organogenesis), or the continuous division of a somatic cell into the embryo and then into the embryo forming a complete plant (direct somatic embryogenesis). In addition, both conditions may occur after a certain period of callus, proto-callus or cell suspension formation (indirect regeneration). In this case, some temporary or hereditary variations may occur. Finally, plant regeneration can occur directly or indirectly from cells containing half of the normal chromosome number. In this case, haploid plants with half the chromosome number of the donor plant can be obtained, which are generally sterile.

Micropropagation can be defined as obtaining new plants from plant parts (embryo, seed, stem, shoot, root, callus, pollen grain in a single cell, etc.) taken from all plants that have the potential to form a complete plant, in artificial basal media and under aseptic conditions (Mansuroğlu and Gürel 2001). It is possible to produce all plant species using *in vitro* propagation techniques if the appropriate nutrient requirements, hormone, and culture requirements of the plants are known enough (Hartman and Kester, 1975). *In vitro* propagation of plants is named according to the explant used (embryo, meristem, anther, cell or protoplast culture, etc.). However, methods such as the single-node method, axillary branching, regeneration of adventitious shoots or buds, and plant regeneration from callus, cells and, protoplasts are mostly used in propagation. The first study of micropropagation, also known as dense clonal reproduction, was carried out in 1902 by the botanist Haberlandt, who tried to prove that a complete living thing could be recreated from a single cell. Haberlandt placed the isolated plant cells in the basal medium. Although living cells in the basal medium increased their volume 11 times, they could not multiply. Studies following Haberlandt attributed this failure to the fact that the hormones necessary for cell division, development, and differentiation were not known in those years.

The importance and advantages of micropropagation in terms of plant breeding and genetics can be listed as follows:

a. Obtaining plant material free from diseases and pests

b. Providing the following benefits in mass production:

- phenotypic and genotypic similarity (homogeneity) in plants produced
- shorter culture time than conventional methods
- easier production of hard-produced species
- rapid production of selected/superior genotypes
- less rootstock use in production

c. Obtaining new cultivars/genotypes due to somaclonal variation

Despite all these benefits, micropropagation requires trained personnel, special laboratory conditions, and continuous research. Therefore the success of the applications is largely limited by economic factors. In many studies, it gives superior results to traditional methods or is at a level that can compete with these methods. In many studies on both herbaceous taxa (ornamental plants, medicinal and aromatic plants, etc.) and woody taxa (fruit trees, forest trees, branches, etc.), *in vitro* plants produced more shoots and more flowers than those propagated by traditional methods. In addition, they were found to be 2-4 weeks earlier than the normal time, commercially uniform (Buisman, 1985; Lisiecka, 1988; Osiecki, 1988; George and Debergh, 2008; Brassard et al., 2013; Sezgin, 2018; Kapdan and Sezgin, 2021).

Mutations, organogenesis and somatic embryogenesis, loss of regeneration abilities of organs and somatic embryos, rooting problems (in shrubs and woody plants), accumulation of toxic compounds in the basal medium, contamination, and difficulties in transferring plants from culture tubes to soil are the main problems that arise in commercial micropropagation. In this sense, productivity, quality, adaptation to different conditions, and resistance in plants are determined by the effect of genotype and environmental factors. Combining these factors consistently in the best way ensures a more economical production (Sezgin 2009). With *in vitro* techniques, many kinds of plants have been reproduced quickly and without problems for years. Gelling agents (agar, gelrite, clerigel, etc.) traditionally used in plant tissue culture have a wide range of uses, thanks to their low cost. Although the propagation of plants with tissue culture is expensive, when automation and robotization techniques that reduce the labor force are used, abundant plants can be economically obtained in a short time. In order to reduce this workload and at the same time increase the amount of reproduction and plant quality, “Plant Tissue Culture Applications in Liquid Medium-Broth Medium” or in other words; Studies are carried out with the principle of immersing the plant material in the liquid growth medium in short time intervals. Plants propagated and regenerated by in vitro techniques are propagated in liquid medium in bioreactors without adding solidifiers to the basal medium. Plants in bioreactors are able to benefit from the basal media at the highest level by passing through the stagnant stage, immersion stage and aeration stages, thanks to automation.

1. WHAT IS A BIOREACTOR? HOW DOES IT WORK?

Biotechnology, as a subject, contains many "multidisciplinary" independent disciplines. Many professional groups such as biotechnology workers, biochemists, microbiologists, geneticists, molecular biologists, cell biologists, botanists, agricultural engineers, virologists, analytical chemists, control engineers, electronic engineers, and computer engineers can be included in this list. In addition, this list can be expanded by adding economists, managers, and finance professionals responsible for marketing a new technique, and lawyers responsible for patenting a new product. In this context, biology science, and engineering science should be considered together to realize intensive and mass production.

Bioreactors are defined as devices that enable biological processes to be carried out under controlled environments and application conditions (pH, temperature, pressure, feed and waste environment, etc.). One of the most important issues in bioreactor design is mass transfer. If the mass transfer is not at the desired level, it is possible that the tissues produced cannot be fed sufficiently. First of all, bioreactors must continuously deliver nutrients (glucose and amino acids), biochemical factors, and oxygen to the cells, ensure the diffusion of various chemicals into the interior of the scaffold, and continuously remove the by-products of cell metabolism such as phenolics. In addition, very important parameters such as pH, oxygen ratio, and nutrient amount can be controlled depending on the efficiency of mass transfer. Mass transfer in bioreactors can be accomplished by passive diffusion or direct perfusion. Oxygen transport by diffusion is only effective down to cell layers 100-200 µm deep. Therefore, perfusion undoubtedly ensures that the transfer takes place more effectively (Riet and Tramper, 1991; McDuffie, 1991; Sota et al., 2021). Another important point in the design of bioreactors is that the reproducibility and reliability of the desired product can be ensured on a large scale for clinical studies. At the same time, aseptic conditions must be provided in the long term to follow the desired tissue-specific functions in bioreactors (McDuffie, 1991; Monja-Mio et al., 2016; Sota et al., 2021).

The working mechanism in a bioreactor works with the logic of a temporary immersion system, in other words, these systems represent bioreactors used in plant biotechnology. Cloning high-quality materials using *in vitro* propagation methods, which have several advantages over traditional propagation techniques, may force the manufacturer with an unpredictable and costly production technology in micropropagation. Techniques currently in use require the preparation of plant tissues by hand under aseptic conditions, as well as semi-solid media and a large number of containers. In addition, due to the depletion of the nutrient medium and the limited continuous growth and proliferation of tissues depending on the volume and dimensions of the culture container, subculturing should be done at intervals of 4-6 weeks and the plant material should be transferred to a fresh medium (Maene and Debergh, 1985). Some disorders may occur in automation due to the inertness of the agar products. The high costs of production often necessitate the use of micropropagation technique, which allows intensive and clonal propagation in plants of commercial or scientific importance (Sluis and Walker, 1985; Simonton et al., 1991). Losses during the acclimatization stage and stem and root hyperhydricity are other major costs (Reuther, 1985). Automating production procedures, using new technologies and implementing appropriate conditioning protocols, will pave the way for commercial application of micropropagation of various species (Kitto, 1997).

Debergh (1988) and Aitken-Christie (1991) stated that the use of liquid media in automation in plant production is an ideal environment for reducing production costs. Liquid culture systems can provide an easy-to-control production method in this regard. The culture conditions can be easily renewed without any vessel change in the subculture stages following the initial stage. After the culture stage of the plants is completed, it can be done more easily because the container cleaning, microfiltration and sterilization stages are passed. Transfer times can also be reduced by using much larger vessels compared to culturing in semi-solid media. There are many successful studies that were cultured in liquid media instead of semi-solid basal media and when the plant was reproduced by tissue culture methods. For example; in peach (*Prunus persica* L.) (Hammerschlag, 1982), in wheat (*Triticum aestivum*) (Jones and Petolino, 1988), in cotton (*Gossypium hirsutum*) (Gawel and Robacker, 1990), in coffee (*Coffea arabica*) (Etienne-Barry et al., 1999), in sugar cane (*Saccharum* spp.) (Lorenzo et al., 1998), in oak (*Quercus* spp.) (Mallón et al., 2012), in apple (*Malus sylvestris*) (Sota et al., 2021).

1.1. Micropropagation Systems In Liquid Culture Inside The Bioreactor

Bioreactors were originally invented for the purpose of carrying out bacterial cultures. It is not actually intended for use for micropropagation or cell culture. The advantages of micropropagation in the liquid basal medium are generally considered to be solutions to technical problems such as asphyxia, hyperhydricity, explant preparation, and the need for a large number of equipment used at this stage. In addition to culture stage supports such as paper bridges, plant-based blocks, etc., different methods have been developed to be used in the solution of these problems (Connor and Meredith, 1984; Hamilton et al., 1985; Maene and Debergh, 1985; Weathers and Giles, 1988; Etienne et al., 1991; Tisserat et al., 1993; Smith and Spomer, 1995; Wataad et al., 1997).

The basic principle of the Temporary Immersion System is to immerse plant material in liquid medium for short periods of time. For micropropagation, a system similar to fog bioreactors, preferring temporary contact of plants and liquid media instead of permanent contact, was used (Etienne and Berthouly, 2002).

1.2. Temporary Immersion Systems (TIS)

In order to benefit from the positive effects of liquid growth media and aeration, Harris and Mason (1983) designed machines that work with tilting logic. In the following studies, the principles of systems operating with the "semi-automatic temporary immersion principle" are also stated by Teisson et al. (1999):

- avoiding the negative effects of continuous immersion
- effects on growth and morphogenesis,
- ensuring adequate oxygen transfer;
- ensuring adequate mixing;
- limiting cut-off levels;

- automation and enabling sequential environmental changes;
- reduction of contamination;
- low cost.

TIS differ depending on the size of the culture vessel, the type of culture medium, whether the timer is simple or computer-controlled, the type of pump used (peristaltic or air pump), or the mechanical mobility of the vessel used during the discharge phase. In TIS, the recycling of the basal medium or the presence of a second culture tank in the middle makes the system different from other systems. The common features of TIS are that they have larger containers than traditional culture vessels, and that they are transparent and autoclavable (Figure 1). Long-term subculture can be done in these systems, which are simpler to use compared to conventional bioreactors. Temporary immersion culture systems can be programmed to provide partial or complete contact between the explant and the liquid medium.

Figure 1: Temporary immersion systems culture and media vessels (SETIS™)

Temporary immersion systems in *in vitro* plant propagation were determined according to their application and divided into 4 different groups. These are;

• The principle of tilting and rocker arming machines;

- The principle of complete immersion of plant material by filling the basal medium;
- Mechanism system providing partial immersion and liquid medium supply;
- Full immersion system with driven transfer of liquid medium, without basal medium replenishment by pneumatics.

The most basic operation logic of TIS is shown in the Figure 2.

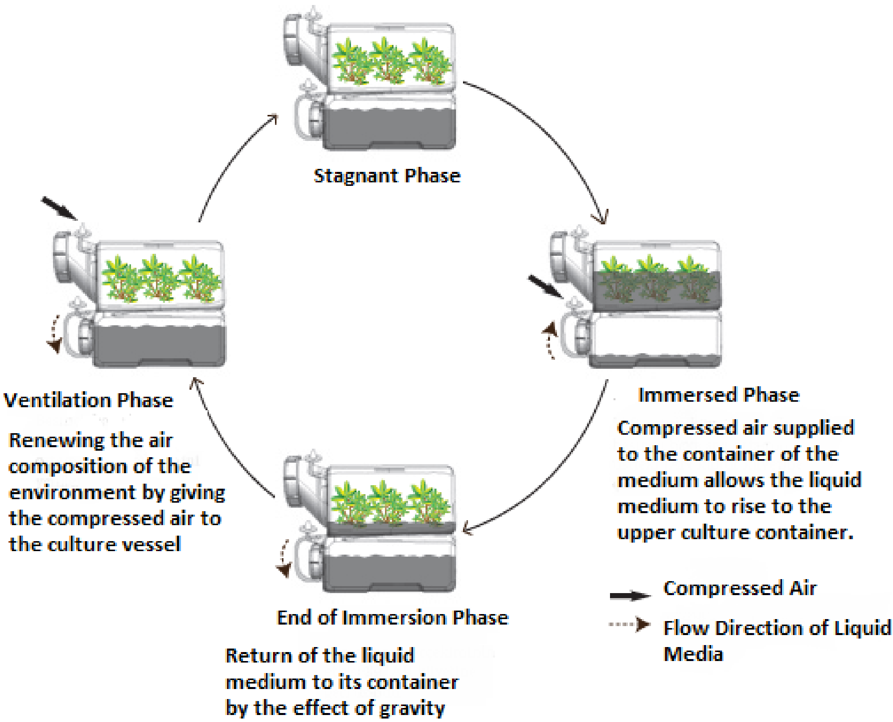

Figure 2: Demonstration of the Temporary Immersion System's working principle (SETIS™).

In the study of Harris and Mason in 1983, there were two different machine suggestions. Among these, are the tilting machine, which operates by tilting the basal medium in an Erlenmeyer at various degrees, and the rocker machine which works with the principle of rolling wide-mouthed jars by tilting them. Tisserat and Vandercook (1985) developed a large-volume culture chamber that can be refilled with sterile and fresh medium after periodically emptying. This automatically working system stands out as a useful method for *in vitro* plant culture in the long run. Simonton et al. (1991) conducted culture studies with the aid of a computer-controlled pump that applied liquid medium to plants intermittently. Explants were placed in the container by sitting on a polypropylene sieve. Alvard et al. (1993) investigated new temporary immersion systems in their study. The system they used was simple and easy compared to the previous ones. It provides a complete renewal of the culture medium with an aeration mechanism that pushes the liquid basal medium towards the plant material. Thus, it ensures the contact of the explant with the liquid medium. In such systems, the basal medium is pneumatically transferred from one tank to the other tank containing the plants. The high pressure is balanced by a solenoid valve or programmable compressor. In this way, the time and duration of the application are determined (Figure 3).

Figure 3: Temporary Immersion System (TIS) (SETIS™) a-b) plants in culture and media containers c) specialized shelves for SETIS™ bioreactors (Sezgin and Durak, 2015).

In these systems, the culture medium must be renewed at least every 4-6 weeks as it does not contain an extra fresh medium tank. Since there is no need to renew the container with the plant material, this regeneration process can be done quickly.

1.3. The Effect of Temporary Immersion System On Efficiency of *in vitro* Techniques

When contemplating commercial and large-scale use of temporary immersion systems connected to a specific automation system, it is necessary to make extensive measurements of the development, production, and quality of the cultivated plant material and to compare them with materials produced by conventional propagation systems. The cost-benefit relationship of the method should always be considered.

Shoot and nod culture

One of the first studies on this subject was the result of Aitken-Christie and Jones (1987) subculture of shoots on semi-solid media performed every 4 weeks in *Pinus radiata*, emphasizing that TIS promoted shoot proliferation more. TIS provided 18 months of continuous shoot growth and monthly harvesting without the need of transferring plant material. The shoots obtained by temporary immersion in the basal medium were of higher quality than those obtained in the semi-solid medium. Alvard et al. (1993) stated in their study that the application of liquid medium in banana (*Musa* spp.) explants had a strong effect on the development and proliferation of shoot tips. TIS have provided very successful results in this plant. Lorenzo et al. (1998), *Saccharum* spp. (sugar cane), they stated that the TIS significantly affected shoot formation and length. The shoot formation rate (23.9 shoots/30 d) was exactly 6 times higher than the standard protocol (3.96 shoots/30 d; Jiménez *et al*., 1995). Likewise, Escalona et al. (1999) used the same type or exactly the same one bioreactor for the shoot tip culture of pineapple (*Ananas comosus*) and obtained an increase of 300 to 400%. Up to 5000 pineapple plants were obtained from one plant. In coffee (*Coffea arabica* and *C. canephora*), TIS restricts propagation by micro cuttings on a semi-solid medium due to the slow growth rate of orthotropic shoots. The propagation rate with TIS increased approximately 6 or 7 times in 3-month periods (Söndhal *et al*., 1984). In grapes (*Vitis vinifera* L.), immersion intervals of 30 seconds or longer obtained seven times more shoots than those kept in a semi-solid medium (Harris and Stevenson, 1982; Harris and Mason, 1983). Similar results in shoot numbers were also obtained in *Arctostaphylos uva ursi* (L.), *Amelanchier alnifolia* Nutt., and fuchsia (*Fuchsia hybrida*) plants (Stevenson and Harris, 1980; Kada et al.,1991; Hunkova et al. 2017).

Somatic embryogenesis

In somatic embryogenesis, unlike organogenesis, the plant is obtained without any cutting process during the propagation stage. In this method, plant regeneration takes place in four stages: (a) promotion of embryogenic culture, (b) production, (c) maturation of somatic embryos, and (d) development of plants (Arnold et al. 2002).

There are few studies on the use of temporary immersion in somatic embryogenesis in the induction phase. The use of TIS is mostly used in the stages of proliferation, maturation, and germination of embryos. However, Snyman et al. (2011) used a direct bioreactor to initiate embryogenic culture in *Saccharum* spp. where they used leaf discs as explants, and Sankar-Thomas et al. (2008) used hypocotyl segments in *Camptotheca acuminata*. TIS were effective in the proliferation of embryogenic callus. Marbun et al. (2015) in their study on *Elaeis guineeensis*, achieved a seven-fold increase in the first embryogenic callus by dipping at a frequency of 3 minutes/3 hours. Maturation, development and high yield of somatic embryos have been observed using various types of TIS (Etienne et al. 1997a; Etienne-Barry et al. 1999; Tahardi et al. 2003; Niemenak et al. 2008). TIS also had positive effects on the germination of somatic embryos (Etienne et al. 1997b; Etienne-Barry et al. 1999; Tahardi et al. 2003). Etienne et al. (1997b) in their study on *H. brasiliensis*, attributed the rate of root growth (60%) and epicotyl formation (35%) to the temporary submersion system. Embryos produced by TIS in *C. sinensis* occurred more in quantity (25.4%) than those produced in the semi-solid system and reached a higher germination rate (46.4%) in terms of germination rate. Similarly, the transformation rate of embryos germinating into plants is higher in TIS-derived embryos (87.7%) than in semi-solid system-derived embryos (38.3%) (Tahardi et al. 2003). Gatica-Arias et al. (2008) also moved up the transformation period of embryos into plants 2 weeks ahead of the semi-solid medium in *C. arabica* in their study. They conducted another study in *C. canephora* where there was a 95% transition from the torpedo stage to the cotyledon stage with TIS (Ducos et al., 2007, 2010).

Parameters affecting the efficiency of TIS

The most important reason why temporary immersion systems are so effective is that the entire surface of the tissue in the ventilation and liquid nutrient medium is in an intermittent contact. In other liquid culture procedures, these two features are often not simultaneously applied. In temporary tissue immersion culture systems, immersion time is very important as it determines nutrient uptake and control of hyperhydricity.

The length used for immersion varies considerably from species to species. For example, long-term immersion (1 hour in every 6 hours) was effective for tuberization of potatoes, while very short-term immersion (1 minute in every 12 hours) increased somatic embryogenesis in *C. arabica* and rubber (Etienne et al., 1997a, 1997b), and it was also efficient in grapevine shoot propagation (30 seconds in every 30 seconds) (Harris and Mason, 1983). In the studies comparing the immersion time and frequency, an increase in the number of shoots was obtained when the immersion time was kept constant and the frequency was changed, while the quality shoot development was obtained in the experiments where the frequency were extended. In experiments in which immersion times were shortened, it was stated that the resulting stress weakened the plant by drying out the shoots (Aitken-Christie and Jones, 1987; Krueger et al. 1991; Berthouly et al. (1995). In the study performed in *Coffea* spp., changing the immersion time in the production of somatic embryos greatly affected the result (Teisson and Alvard 1995).

Success in TIS is achieved through an optimization study and differs according to the plant type, plant variety, in vitro propagation method used and the desired goal.

The volume of liquid medium and container

Liquid media volume should be optimized in basal media of temporary immersion systems without regeneration. Lorenzo et al. (1998), in their study on *Saccharum* spp., medium volume increased the number of shoots up to 10 times but did not provide any positive development in shoot lengths. Using the same temporary immersion system, Escalona et al. (1999), when they used pineapple shoot growth as 200 ml/explant, they obtained the optimum basal medium volume and emphasized that larger volumes caused a decrease in the growth rate. In all temporary immersion systems, the volume of the vessel used, and hence the space remaining at the top is much larger than in vessels used for conventional in vitro propagation methods. In addition, the amount of product can be increased by easily adapting the containers with varying volumes of 1 to 20 liters to the system.

Aeration or ventilation

The presence of bubbles in the container plays a growth-promoting role in aeration. However, partial immersion of the explant into the medium will not be sufficient for ventilation. Therefore, it is clear that temporary immersion systems are the most effective culture system. Alvard et al. (1993) clearly demonstrated that the lack of air in the liquid culture medium is an important limiting factor in the growth of banana explants. In systems using the pneumatic drive of the basal medium, forced ventilation occurs during immersion, which leads to complete regeneration of the culture atmosphere. According to Monja Mio et al. (2016) gas exchanges in such a system primarily occur during immersion and result directly from the air pump and indirectly from the movement of the liquid. The relative humidity that occurs in the container during forced ventilation can also be considered as an acclimatization phase for the plants to survive in the acclimatization phase or in ex vitro conditions.

1.4 The Effect of Temporary Immersion Systems on Production Quality

It is emphasized in the production studies carried out in the TIS system that although the shoots develop quite frequently and abundantly, there is some reduction in leaf size compared to the classical in vitro propagation methods (Tisserat and Vandercook, 1985; Krueger et al., 1991; Escalona et al. 1999; Monja Mio et al., 2016). However, in general, it has been reported that there is a significant increase in the amount of proliferation with the use of TIS compared to semi-solid media in the same studies. This differs significantly from semi-solid media at all stages as well, such as stimulation, development and regeneration of somatic embryos. (Etienne et al., 1993; Escalant et al., 1994; Cabasson et al., 1997; Etienne et al., 1997a, b; Etienne–Barry et al., 1999; Monja Mio et al., 2016). Immersion systems have many positive effects on somatic embryos, reaching a density of approximately 1600 embryos per 1 liter bioreactor, elongated embryonic axis, up to the two-fold increase in fresh weight, and a stimulating effect on the germinated somatic embryo morphology at the cotyledon stage. These morphological changes also have a positive effect on plant transformation and growth rates.

In micropropagation in a liquid culture medium, it is quite common to see a state of hyperhydricity while promoting growth by increasing nutrient uptake. Here, the constant contact of tissues with the liquid medium stands out as the source of this undesirable condition (Ziv et al., 1983; Hussey, 1986; Debergh, 1988; Watt, 2012). Vitrified and waterlogged tissue appearance of plant tissues causes irregular growth of the shoot system. More specifically, different degrees of morphological and physiological disorders are observed in the leaves that abnormally develop (Ziv, 1995). In their study, Aitken-Christie and Jones (1987) emphasized that regular aeration in the culture medium and immersing the explants in a liquid culture medium at regular intervals would be effective in reducing hyperhydricity. It is known that these two features are used together in many temporary immersion systems.

Losses during acclimatization are a serious problem in conventional micropropagation. Air conditioning in the temporary immersion systems can be successfully completed. Most studies also highlight that plants grown with TIS are much more successful in surviving the ex-vitro acclimatization stage than those grown in semi-solid and liquid media. Ex vitro acclimatization and rooting under semi-industrial conditions on pineapple, *Saccharum* spp., Eucalyptus shoots was a more productive and routine process for shoots produced in the temporary immersion system (Lorenzo et al., 1998; Escalona et al., 1999; Etienne and Berthouly, 2002; McAlister et al., 2005; Watt et al., 2006).

1.5. Costs in Temporary Immersion System Culture production

Considering the initial and installation costs of TIS in the first place, it is normal for this situation to be a bit too much for the manufacturer. However, many studies confirm that the yield in the future will be higher than expected with the use of a liquid medium culture procedure for micropropagation. Lorenzo et al. (1998) reported that temporary immersion reduces overall costs by 46% when compared to the standard procedure for the propagation of *Saccharum* spp. in semi-solid media. This gain was primarily obtained from the reduction of the labor force and space savings. Escalona et al. (1999) stated that the use of temporary immersion, where they used shoot tips as explant source in pineapple, increased the number of shoots up to 100 times in the first month of the study. With the protocol they used, production costs per pineapple plant were reduced by 20% compared to the traditional method in liquid medium. According to the authors, the key parameters of this success are reduction in the number of containers used and material handling, the fact that explants are not repeatedly processed in the subculture stage after the initial stage, and minimized and almost eliminated contamination. In the study of Aitken-Christie and Jones (1987), long-term care of *Pinus radiata* shoots through medium supply led to a reduction in labor costs, which positively affected the preference for automation and resulted in a reduction in cost. This is also the first report of a method for propagating shoots for up to 18 months without subculturing. Due to this harvest rate, this system turned out to be approximately 7 times cheaper than the normal method for subculturing *P. radiata* shoots in semi-solid media. Somatic embryogenesis, late maturation, and germination stages are the most expensive methods because of the amount of processing required. Temporary immersion bioreactors are actively used in somatic embryogenesis studies and the germination of these embryos. Production and germination using a direct immersion bioreactor and sowing germinated embryos under ex vitro conditions provided an advantage of 13% and 6.3%, respectively, in terms of space requirements over conventional conditioning of plants grown in semi-solid media (Etienne–Barry et al., 1999; Teisson et al., 1999; Monja Mio et al., 2016).

2. CONCLUSION

In many plant species, the positive effects of temporary immersion systems are clearly seen at every stage of somatic embryogenesis studies as well as shoot, meristem, node and internode propagation. In terms of plant growth and reproduction rates, it is mostly successful compared to semi-solid media. It seems to be highly preferable for plantlets that have passed the regeneration stage. Good results have also been obtained in terms of acclimatization of the plant material in the temporary immersion. The survival rates and stability of the plants at the nursery stage have been enhanced by these systems. It combines the advantages of maximum gas exchange in solid culture media and increased nutrient uptake of liquid culturing. Explant immersion time and frequency are the most important parameters taken into consideration when planning a new study. In addition, productivity is further increased by the control of morphogenesis and hyperhydricity. By optimizing the culture conditions in bioreactors working with basic and simple logic, new information can be obtained on many subjects on the developmental physiology of the plant. In addition to micropropagation, it is also possible to investigate different metabolic processes with temporary immersion methods. This may be informative on the hyperhydricity and inherent constitutive water status of somatic embryos and callus. There are not enough studies on secondary compounds and phenolics that may occur during culture in temporary immersion systems and their removal from the environment. Thanks to the TIS, lower labor costs and less shelf space requirement and consequently significantly lower production costs have been achieved. Significant reduction in the production cost of in vitro plants will enable the easy application of somatic embryogenesis, microtuberization and shoot proliferation techniques with TIS and will pave the way for different studies on more plants.

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CHAPTER 4

EFFECTS OF ALTITUDE ON FRUIT GROWING

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INTRODUCTION

The most important factor affecting the productivity of agricultural activities is the suitability of ecological factors. The variability of features such as latitude, proximity to the water mass, topography, and altitude affect the climate and are effective in different climatic characteristics between regions. Altitude is expressed as the vertical distance of any place from sea level. When the effects of other factors affecting the climate are ignored, it is observed that there are great changes in climatic characteristics depending on the change in altitude.

Due to the thickness of the atmosphere layer and the greater angle of incidence of the sun's rays, among the regions located at the same latitude, those with lower altitudes have a higher atmospheric temperature than the others. It is reported that for every 100 meters of altitude increase, the atmospheric temperature decreases by 1 ºC in dry weather and approximately 0.6 ºC in humid weather (Körner, 2007). In low-altitude places where the air temperature is higher, the water-holding capacity of the air increases. As a result of increased evapotranspiration on the surface of the water and in living things, depending on the hot air, the relative humidity of the air increases as the water holding capacity increases (Körner, 2007). As the altitude increases, the decreasing temperature reduces evaporation from the water surface and the water holding capacity of the air. Thus, the humidity of the environment decreases (Körner, 2007). The lower atmospheric temperature due to the increase in altitude causes the air to cool. Condensation occurs due to the cooling air, so the number of precipitation increases in places where the altitude is high (Körner, 2007). In parallel with the increase in altitude, light intensity and radiation increase (Körner, 2007; Bais et al., 2018; Derebe et al., 2019). With the increase in altitude, decreases in the levels of gases in the atmosphere are observed. This situation causes the atmospheric pressure to decrease (Körner, 2007). The air expands and rises at low altitudes, becomes heavier at higher altitudes, and sinks to the bottom. This situation, which causes the formation of different pressure centers, provides the formation of air movement between these centers. The high-pressure differences in places with high altitudes increase the wind activity in these regions (Körner, 2007).

Metabolic and physiological events in plants are regulated under environmental conditions. For this reason, in parallel with these changes in climatic characteristics, plants show changes in their phenological, morphological, and chemical properties. Therefore, this study investigated what and how climate change due to altitude change affects fruit growing in light of the existing literature.

1. Effect of Altitude on Phenological Characteristics

The altitude effects the maintaining of the garden, thus effects the fruit growing. High or low altitudes significantly affect phenological events in fruits. High altitudes can be colder most of the year and show little photosynthetic activity. In addition, the growth period at these altitudes is limited by temperature. In addition, the microclimate effect can be seen more severely at high altitudes (Inouye & Wielgolaski, 2003). It has been reported that cold air currents at high altitudes can cause delays in bud burst and flowering (Lynov, 1984). Air temperature, altitude, and topography were determined to be the main variables of bud burst and leafing dates, with delays ranging from 2.4 to 3.4 days at 100 m (Pellerin et al., 2012). When we examine 20 years of data on the flowering, leaf opening, and fruit ripening stages of four woody species in southwestern and central Slovakia, a lag in all phenological stages was identified with increasing altitude (Babalova et al., 2018).

Many studies have been conducted on the effect of altitude on different phenological periods of fruits. When the effect of altitude on the morpho-phenological parameters of *Juglans regia* L. was examined in six different regions, flowering started in the first week of April in the region with the lowest altitude (1580 m) and in the first week of May in the highest region (2250 m). Leaf formation started in the third week of April in the lowest region and in the second week of May in the highest region. The transformation of flowers to fruit began in the last week of May in the lowest region and in the first week of June in the highest region (Wani et al., 2014). Significant differences were observed in the phenological history of Hayward and Matua kiwi cultivars at different altitudes. Generally, kiwi varieties grown at an altitude of 20 m sprouted 7 days earlier than those grown at 610 m and shed their leaves 9-10 days later. There was a one-week difference between the full flowering dates, and the low-altitude regions bloomed as early as a week (Zenginbal & Özcan, 2018).

Regarding the effect of altitude on the phenological properties of apricot (*Prunus armeniaca* L.) fruits, flowering and ripening times were significantly affected. Flowering and fruit ripening was delayed by 3.3 and 7.1 days, respectively, for each 100 m altitude increase. Delay in flowering in high altitude areas has been associated with decreasing temperature. Late flowering in high-altitude regions where spring frosts are experienced has also been stated as an important factor in protecting flowers from damage caused by spring frosts (Naryal et al., 2020). The phenological stages of 'Hongdeng' and 'Van' cherry cultivars were followed in the subtropical climate (Shanghai), and temperate climate zone (Qingdao) in China, the ones grown in the subtropical climate had started to bloom earlier than those grown in higher temperate climate. These cultivars have gruit growing intervals as 38 days ('Hongdeng') and 51 days ('Van') in Qingdao, and 29 days ('Hongdeng') and 45 ('Van') in Shanghai. It has been noted that cherries began defoliating in Shanghai in December, while defoliation began in Qingdao in November (Li et al., 2010).

It was determined that 'Tulameen' and 'Polka' raspberry varieties grown in two mountainous regions entered earlier leafing, flowering, and fruit ripening periods at lower altitudes (570 m) compared to plants at higher altitudes (1040 m). The 'Tulameen' variety started flowering on May 15 at low altitude (29 days) and on May 25 at high altitude (27 days), with a delay of 11 days according to different locations. The 'Polka' variety started to bloom on June 25 at low altitude (57 days) and on July 1 at high altitude (67 days). A delay of 7 days was also detected in this cultivar. In terms of maturation dates, the 'Tulameen' variety started to mature on June 14 at low altitude (27 days) and on June 22 at high altitude (26 days) with a delay of 9 days. It was noted that the 'Polka' cultivar started to mature on July 23 at low altitude (55 days) and on August 5 at high altitude (52 days) with a delay of 14 days (Zejak et al., 2021).

Phenological analysis of trees growing at 965 m and 1310 m altitudes for coffee showed that, the differences began to become evident in the months after February on ripening levels, and there was a slower ripening at 1310 m altitude. So, it was observed that altitude extended the generative period of coffee by one month (Santos et al., 2018).

With the recent climate warming in Switzerland, it has been determined that the spring phenology in species such as apple, pear, hazelnut, and linden shifts more with altitude, with a colder climate. The start of the pre-season was delayed by an average of 2.67 days per 100 m altitudes, and the end of the pre-season, i.e., the average start date of events, by 3.05 days per 100 m altitudes. The length of the preseason increased slightly with altitude (0.44 days on average at 100 m), but the trend varied greatly between species (Güsewell et al., 2017).

In Colombia, pineapple guava fruit was harvested 99 and 141 days after planting, respectively, at two locations at different altitudes (1,800 and 2,580 m). It was concluded that phenological stages of pineapple guava fruit was affected by altitude and weather (Parra-Coronado et al., 2015).

When the flowering phenology of *Olea europaea* L. was examined, it was revealed that the dates of the beginning of the blooming period depends on altitude. Olive groves between 200-700 m were started blooming first, while the ones that grove above 700 m were the last. And they started blooming 7-10 days later (Aguilera & Valenzuela, 2009). When we look at the influence of 6 locations between 450 m and 1250 m on the spring phenology of olive varieties in Argentina, the opening of the first leaves occurred after the opening of flower buds at lower latitude locations, but before the opening of flower buds at cooler high locations. 26 days of disparity in full bloom was detected between the locations. At the same latitude, flowering delayed 16 days at 1246 m when compared to 450 m. Generally warmer and lower altitude areas showed earlier blooming time than higher altitude areas (Hamze et al., 2022).

As a result of time series analyses for phenological events (1980–2009), an earlier phenological beginning was observed at lower altitudes in nine locations centered in three German cities. Altitude effects are quite significant on phenological mean dates, ranging from 1.34 days (100 m)-1 for *Fagus sylvatica* to bloom and 4.27 days (100 m)-1 for *Corylus avellana* to bloom (Jochner et al., 2012).

2. Effect of Altitude on Morphological and Pomological Features

The return on investment in fruit cultivation is only possible with the production of sufficient and high-quality fruit in the right tree form. For this reason, economic and sustainable production is only possible if plant development is directed adequately and correctly. Although it is known how important the cultural processes applied for plant development are, the altitude of the growing region above sea level affects the growth and development of fruit trees as it changes the climatic characteristics. It is known that the same genotype while adapting to regions with different climatic characteristics, shows great morphological changes (Ichie et al., 2016; Tfwala et al., 2019).

At high altitudes, falling air temperature and relative humidity limit plant growth by causing a narrowing of the vegetation period. The adaptation mechanisms of the plants against the more severe climatic events in these regions effectively restrict vegetative development. Increasing wind speed and decreasing relative humidity force a reduction in leaf area to keep plants' water loss to a minimum. Due to the intensity of short wavelength rays at high altitudes, plants generally tend to harden and feather in the leaves to protect them from high temperatures and reduce water loss (Fischer et al., 2016). Studies have reported that the number of stomata in the leaves can change with the intensity of light and humidity. It is expected that the stomatal density will decrease with the reducing in the amount of moisture due to the increasing altitude (Pato & Obeso, 2012). UV light intensity explosion due to the increase in altitude causes a decrease in shoot length and, thus, plant height.

It has been reported that plant growth, chlorophyll amount, and leaf area are higher in blueberry fruits at an altitude of 1000 m than at an altitude of 2000 m (Pato & Obeso, 2012).

Depending on the increase in altitude in apples, leaf structure, and fruit quality parameters showed significant change trends. That is, leaf parameters such as leaf thickness, and cuticle thickness increased gradually. Leaf length, width ratio, specific leaf area, stomatal length-width ratio, and the ratio of the upper and lower epidermis to leaf thickness decreased gradually; similarly, while fruit parameters such as fruit shape index and firmness increased, on the contrary, hue angle decreased (Wen-wen et al., 2014).

It has been reported that leaf lengths vary depending on altitude, and fruit inner diameter and fruit inner weight increase as altitude raise in walnuts. Still, it has been emphasized that these parameters are related to yield (Wani et al., 2014). It has been observed that the total leaf area and fruit volume in apples increase depending on the rising of altitude (Melke & Fetene, 2014). Aslantaş and Karakurt (2009) reported that apple cultivars decreased in the number, length, and width of branches due to the shortened vegetation period at high altitudes. As a result of the selection study carried out in tea, the shoot length of the ‘TRI2023’ genotype increased due to the increase in altitude, but the ‘TRI2025’ selection gave the opposite reaction (Balasuriya, 1999). In bananas, plant length and cluster weight decreased depending on the cultivar and height at different altitudes (Sikyolo et al., 2013).

Generally in studies, it was stated that, hormones synthesized in fruits govern cell division. Cytokinin, gibberellin, and auxin hormones, which are effective in the division, play an important role in forming the final shape of the fruit. Since the amounts of these hormones change at different temperatures, the effect of altitude on the amounts of these hormones is important. In general, in regions and years where the temperature is high, the cell division process is negatively affected, and the final shape of the fruit is affected by different altitudes depending on the type and variety grown (Karaçalı, 1990). In addition, fruit shape varies depending on altitude. It is stated that the fruit shape is longer in the regions where the flowering period is cooler, and the fruit is more rounded in the warmer regions (Atay et al., 2009). A study conducted on the ‘Hayward’ kiwi variety stated that fruit weight, width, and length decreased as the altitude increased (Bostan & Günay, 2014). It has been reported that the fruit weight increases with the increase in altitude, but there is no change in the hardness value in figs (Trad et al., 2013). It has been reported that the fruit width, length, and weight of the ‘Yıldız’ rosehip cultivar decrease as the altitude increases (Güneş et al., 2017). In walnuts, fruit weight, width, and length were increased depending on the increase in altitude (Büyüksolak et al., 2020). In wine grapes, the increase in altitude increased the fruit width, length, and weight (Regina et al., 2010). While there was no remarkable difference in the parameters examined in guava depending on the fruit weight, diameter, and height, the quality parameters specified at low altitudes increased depending on the intensity of the cultural application (Musyarofah et al., 2020). An increase in fruit flesh firmness was observed in apples due to the increase in altitude (Charles et al., 2018). The study on different varieties and strawberries' altitudes observed that the fruit weight was determined at the lowest value in the highest location (Gündüz & Özbay, 2018). It has been reported that fruit weight, fruit diameter, and firmness decrease with altitude in mandarin, while shell thickness and fruit length increase with increasing altitude (Timilsina & Tripathi, 2019). Some studies demonstrated results that vary according to the variety besides altitudes. The reason for this is thought to increase in the dry matter accumulation in the fruit with the increase in photosynthesis due to the rising temperature and optimum conditions at low altitudes, but this may vary according to the type and variety grown. For example, in a study conducted on the 'Luetta' and 'Leccardina' varieties of chestnut, fruit width, length, and weight decreased with increasing altitude in the 'Luetta' variety, while it increased in the 'Leccardina' variety (Silvanini et al., 2014). Fruit weight increased, and color change accelerated with decreasing altitude in guava fruits (Solarte et al. 2014). Another study on guava fruits reported that fruit weight, length, and diameter increased significantly with increasing altitude, while fruit firmness decreased (Parra-Coronado et al., 2015). In peaches, increasing altitude increases fruit firmness (Karagiannis et al., 2016). In kiwifruit, fruit yield and some fruit quality parameters were reported to be higher and recommended for cultivation at lower altitudes (Pandey et al., 2004). In studies on coffee and banana, it was reported that yield decreased with increasing altitude (Avelino et al., 2005; Sivirihauma et al., 2014).

As the altitude increases, the increasing light intensity contributes positively to the coloration of the fruit peels, on this matter, the factor being the accumulation of dry matter amount. In areas where the temperature difference between day and night is high, dry matter accumulation is also high (Tonietto & Carbonneau, 2004). High altitude and south orientation combined with apple fertilization resulted in better-colored fruit. High altitudes also exhibited higher fruit firmness (Singh et al., 2009). It was observed that the redness of the grain (a*) color of pomegranate increased significantly with the increase in altitude (Yaman et al., 2015). The effects of three altitudes (1570, 1720, and 1890 m) on guava fruit quality were investigated. Fruit weight and color development were affected by altitude. It was observed that higher fruit weight was accompanied at low altitudes where high temperature and high radiation predominate, while an opposite trend was observed at higher altitudes (Solarte et al., 2014). It has been reported that the Chroma color value varies depending on the altitude, fruit maturity stage, and fruit variety in blueberries. In the 'Duke' cultivar, Chroma value increased with the increase in altitude at early maturity stages and decreased at advanced maturity stages. The situation was the opposite in the 'Brigitta' cultivar (Spinardi et al., 2019).

3. Effect of Altitude on Chemical Properties

Chemical components, the most important factors affecting the formation of taste in herbal products, are also extremely important in regulating many physiological events. Chemical properties with low heritability are highly sensitive to changing environmental conditions. In parallel with the changes in meteorological parameters, it is reported that the chemical properties of fruit species also undergo major changes (Polat et al., 2020; Fischer et al., 2022).

In high-altitude regions where the vegetation period is short (Inouye and Wielgolaski 2003), phytochemical accumulation remains low because the time required for the maturity of late varieties cannot be provided (Mertoglu et al., 2020b). Conversely, early or mid-term maturity cultivars are reported to show generally good results regarding phytochemical accumulation. Since providing the optimal day-night temperature difference for photosynthesis at higher altitudes increases the assimilation products produced by photosynthesis, the amount of water-soluble dry matter in fruits increases. The fact that fruits harvested at high altitudes have less intercellular spaces also contributes to this situation.

In plum, the ‘Angeleno’, a late variety, was grown both at high (800 m) and low altitudes (200 m), and the harvested fruits were examined in terms of phytochemical properties. The amounts of malic acid, acetic acid, oxalic acid, and ascorbic acid were determined as 7655.8, ppm 2822.4 ppm, 838.4 ppm, and 12.7 ppm at low altitudes, respectively, while these values decreased to 5924.5 ppm, 1937.1 ppm, 564.1 ppm and 12.0 ppm at high altitudes (Mertoglu et al., 2020b). All organic acids, except acetic acid, were detected at higher levels at higher altitudes for the mid-season variety ‘Black Diamond’, which was examined under the same conditions (Mertoglu et al., 2020a). Studies report an increase (Kumar et al., 2019) or a decrease (Gündüz & Özbay, 2018) in the amount of organic acids depending on the increase in altitude. It has even been reported that the cultivars do not exhibit a stable condition in terms of organic acids, and higher values have been observed at both low and high altitudes over the years (Correia et al., 2016). This can be interpreted as plants' phytochemical composition, and accumulation are shaped under the cumulative effect of many factors, and the conditions close to harvest, where accumulation accelerates, are important (Rivera et al., 2017; Edgley et al., 2019). Examining the changes occurring at the genotype level and under which factors are necessary.

Changes in organic acids directly affect titratable acidity and pH properties. In high-altitude regions, where organic acids are generally more abundant, the amount of titratable acidity increases while the pH remains at low levels. In the products to be processed to gain added value, it is desirable to maintain product stabilization and low microorganism activity. In this case, since high acidity or low pH will be advantageous, it can be said that the fruits obtained from cultivars with early or mid-term maturity will be more suitable for the processing industry. For this reason, it may be advantageous if the fruits, which are planned to be stored for a long time, are grown at high altitudes (Faniadis et al., 2010).

The light intensity and the temperature difference between day and night at higher altitudes increase. This situation ensures that phenolic acids, secondary metabolites, are synthesized more during increased photosynthesis (Mditshwa et al., 2013; Gunduz & Ozbay, 2018). In addition, because photosynthesis cannot be performed and the air temperature is not high at night, the destruction of phytochemicals is ensured by staying at lower levels due to respiration at high altitudes. Plants produce more phenolic compounds in the phenylpropanoid pathway in epidermal tissues as a defense agent against the harmful effects of high UV. Josuttis et al. (2010) reported that, phenolic acids increased in strawberries grown in the open and in a UV-blocking plastic tunnel and strawberries grown outdoors and exposed to higher UV. Based on all these situations, it has been reported that phenolic compounds are measured at higher levels in general in fruits harvested at high altitudes (Mansour et al., 2022).

Organic acids and phenolic compounds are chemicals with high antioxidant effects (de Souza et al., 2012). In addition, increasing the UV amount increases antioxidant activity by stimulating plants' enzymatic and non-enzymatic defense systems (Martinez-Lüscher et al. 2013). For this reason, it is reported that fruits are grown at high altitudes generally have higher antioxidant activity (Karagiannis et al., 2016). In different studies, it has been reported that the anti-microbial activities of these fruits also increase (Mertoglu et al., 2020a).

As a result, no matter how important the routine maintenance conditions are in fruit growing, it is seen that the location affects fruit yield, quality, and chemical composition of the fruit variety. At the same time, altitude plays a major role in fruit quality and yield by affecting the morphological, phenological and chemical characteristics of plants, such as bloming time and generative periods, crown structure, leaf width, the number of shots, and chemical content of fruits. High or low altitude has positive or negative effects depending on the type and variety of fruit grown and the care conditions of the plants. Therefore, it is necessary to increase the number of studies on the effects of different altitudes on fruit growing.

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CHAPTER 5

INVESTIGATION OF THE ADVERSE EFFECTS OF TEMBOTRIONE ON TOMATO AND THE POSSIBILITY OF DISPOSAL

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INTRODUCTION

Tomatoes stand out among vegetables due to having one of the highest productions - 186 million metric tons in 2020, in the World (FAO, 2022a). Türkiye, on the other hand, is the 3rd largest tomato producing country in the world after China and India with over 13 million metric tons of production (FAO, 2022b). Such a high amount of tomato production means 41.5% of the total vegetable production in the country (Anonymous, 2022). Tomatoes are grown approximately in every region of Türkiye, are often planted in close or adjacent fields with crops such as maize, beans, paddy rice and sunflowers due to coinciding growing seasons. Herbicides applied to control weeds in these fields can drift into the tomato fields when the necessary conditions are available. One of the most used of these herbicides is tembotrione, [2-(2-chloro-4-methylsulfonyl-3-(2, 2, 2 trifluoroethoxy) methyl] benzoyl) cyclohexane-1, 3-dion)] which controls broadleaf weeds in maize. It acts by inhibiting 4-hydroxyphenylpyruvate dioxygenase (HPPD), which causes chlorophyll destruction by photo oxidation and whitening of the resulting leaf tissue (Corneiro et. al., 2019; Dias et. al., 2019).

Tembotrione reaches off-target plants with the effects of various factors during application to maize areas such as wind, residue in the soil, and improper use of equipment (Alves et. al., 2017; Nordby & Skuterud, 1974). Although the injury of tembotrione on off-target crops is generally in the form of carryover and soil residue in the literature, wind drift simulations has not been studied. Studies in the past shows that, the residue of tembotrione was up to 300 days after application in tropical soils with water deficit, indicating tembotrione may present variable soil persistence (Silva et. al., 2018). However some negative effects of tembotrine were reported on potatoes tuber yield and N, K contents of tubers (Dias et. al., 2019). Additionally some other carryover damages of tembotrione are reported on carrots and beets about loss of yield, quality and physiological disorders (Bontempo et. al., 2016; França et. al., 2016; Eberlein et. al., 1997; Thornton & Eberlein, 2001). The negative effects of tembotrione were initially detected in 2020 on tomatoes which are grown in Kumkale plain of Canakkale as observational.

Many researches carried out to overcome the herbicide caused injuries. Crop injury caused by herbicides can be alleviated by following the guidance of safe use of herbicides. (Kim, 1992; Sharma, 2017). On the other hand, some scientists have tried to find different solutions to reduce the negative effects of herbicides such as use of plant regulators (Grossmann and Schmülling, 1995).

We propose an approach in which plant growth regulators play a leading role in preventing tembotrione-induced damage to tomatoes in this study. As explained above, tembotrione based herbicide drift on tomato plant damages the physiological pathways and some damages may cause. We propose a recovery application which is a combinatorial application of plant growth regulators (NAA, IAA and GA3) and fertilizers to alleviate herbicide injuries.The goal of this study is to determine the effects of tembotrione drift by the wind and to investigate the possibilities of eliminating these damages by using various fertilization and plant growth regulators.

MATERIALS AND METHOD

In this study, Elegro F_1, one of the most widely grown cultivar in Canakkale, Türkiye, was used as a plant material. A replicated field trial was carried out in the research fields of Faculty of Agriculture at Canakkale Onsekiz Mart University, Dardanos, Çanakkale, Türkiye in 2020 growing season. The soil where the plants grown were clay loam structure with 7.80 pH, 0.69 mS/cm, 11.90% lime and 1.12% organic matter. Research was carried out according to randomized block design with 3 repetitions and 30 plants in each repetition. Tembotrione active ingredient herbicide (Laudis® OD66, Bayer Cropscience) was applied on the tomato plants with the help of a sprayer when the plants were in generative stage approximately 30 days after transplanting. The doses used in the study were determined according to the scenario in which 0%, 5%, 10% and 20% of the 20 ml/ha dose suggested on the label of the herbicide was assumed to drift on the tomato plants by the wind. Thus, the doses were arranged to be 0.0 ai, 2.2 ai, 4.4 ai and 8.8 ai. In addition a recovery (R) formula planned to eliminate the tembotrione damages on plants. The recovery (R) formula includes;

1. 50 g NAA + 52 g IBA plant growth regulator (Massplant®) was applied to the plants a week after tembotrione spray for all doses.
2. 15-30-15+2Me N-P-K fertilizer (Poly-feed by Haifa®) 50 kg ha^{-1} applied by irrigation a week after tembotrione spray for all doses.

3. 2 g of GA_3 (Megafill®) dissolved 100 L water and sprayed to the plants 10 days after tembotrione spray for all doses.
4. Isabion (Syngenta®) amino acid 2500 ml ha^{-1} sprayed to the plants 10 days after tembotrione spray for all doses.

Standard cultural practices were utilized and no fertilizers applied to the soil for exact determination of herbicide and R treatments. Yield (kg da^{-1}), yield per plant (kg $plant^{-1}$), relative growth rate (g d^{-1} d^{-1}) (Causton, 1994), fruit weight (g), fruit color (hue° and chroma) (McGuire, 1992), soluble solid content (SSC %), EC and pH in the fruit juice, titrable acidity (TA %) (Karaçalı, 2009; Anonymous, 1968), and vitamin C (mg100 ml^{-1}) (Pearson, 1970) were also determined.

Analysis of variance (ANOVA) and Duncan's Multiple Comparison Test was used to determine the significant differences in each trait. The SPSS (ver. 16.0) statistical analysis program was used for statistical calculations.

RESULTS AND DISCUSSION

When the data were analyzed in terms of yield (kg da^{-1}), statistically significant differences were found between the treatments (Table 1). This difference is significant at the ($p \leq 0.01$) level. While the highest yield was obtained in the 0.0 ai (Control) treatment, the lowest yield was observed in the 8.8 ai application. While 2.2 ai and 2.2 ai + R were in the same group statistically, it was observed that recovery applications increased the yield in all other herbicide applications. It was determined that as tembotrione active ingredient increased, the yield decreased and the herbicide dose was inversely proportional to the yield. The yield loss of the tembotrione-treated group was 42.84% compared to the 0.0 ai group. It is observed that yield values per plant decreased with increasing doses of tembotrione, but single fruit weights increased with increasing doses. Besides the fact that the yield decreases with the increase of tembotrione doses, it can be claimed that flower deformation occurred caused by tembotrine. As proof of this, it can be shown that the average fruit weight increased despite the decrease in yield. This situation reveals that as a result of flower deformation, pollination and fertilization stages of flowers are prevented and less fruit set occurs. In other words, as in fruit cultivation, fruit thinning (e.g. apple) causes to get larger fruits without affecting on the cumulative yield. In summary, when tembotrione reaches non-target tomatoes by wind, it can directly affect yield by causing flower deformations. Although there is no quantitative measurement of this situation in our study, we had similar observations to support this argument (Figures 1 and 2).

When the data were analyzed, yield per plant (kg) determined statistically significant. This difference is significant at ($p \leq 0.01$) level. While the highest value was obtained in 0 ai application, the lowest value was obtained in 8.8 ai + R application. Although 0 ai application was higher than 0 ai + R application, it was in the same group statistically. In the 2.2 ai + R application, a yield decrease of 11.6% was observed compared to the 2.2 ai application (Table 1). An increase of 10.9% was observed in the 4.4 ai + R application compared to the 4.4 ai application. Likewise, the yield per plant decreased by 40.4% in 8.8 ai + R application compared to 8.8 ai treatment. It is determined that the yield per plant decreased as the tembotrione doses increased due to the negative effect of tembotrione on chlorophyll loss. The plant cannot produce enough nutrients and energy for its own needs with photosynthesis. Although there are no studies on the determination of the effects of tembotrione on off-target plants by direct wind drift in the literature, it is seen that the residue effect in the soil has been particularly studied. The presence of tembotrione in soil reduces growth and amount of potato tubers and dry mass of carrots (Dias et al., 2018; Bontempo et. al., 2016). Authors speculated that it might also be possible to have similar negative effects in tomatoes. It should be noted that this yield loss may be due to the mechanism of action of tembotrione which leads to chlorophyll destruction via photo-oxidation and whitening of the resulting leaf tissue.

Table 1: The effect of tembotrione and recovery (R) applications on yield and some quality parameters in tomato

Application	Yield (kg/da)	Yield per plant (kg/plant)	Average fruit weight (g)	RGR (g/d/d)	Fruit length (mm)	Fruit diameter (mm)	Hue°	Chroma	SSC %	EC	pH	TA %	Vit.C (mg/100ml)
0 ai	3455,01 a	1,710 a	140,31 ab	0,0373 b	63,33 a	63,32	38,72	24,85 c	5,37	5,04	4,38	0,56	12,11
2,2 ai	2416,16 ab	1,321 ab	90 bc	0,0324 e	58,62 bc	63,52	37,74	25,84	5,53	4,77	4,45	0,67	12,78
4,4 ai	1975,08 bc	0,978 bc	98,28 bc	0,0319 f	57,83 bcd	59,12	37,15	25,38	5,67	5,14	4,43	0,45	13,11
8,8 ai	791,44 d	0,720 cd	130,21 abc	0,0348 c	55,44 cd	58,72	38,04	27,08	5,86	5,95	4,48	0,72	15,36
0 ai + R	3322,99 a	1,645 a	156,59a	0,0382 a	62 ab	65,18	39,94	26,15	5,57	4,28	4,24	0,67	15,57
2,2 ai + R	2359,22 ab	1,168 abc	83,99 c	0,0340 d	59,26 abc	60,52	38,24	25,79	5,37	4,99	4,35	0,41	9,10
4,4 ai + R	2191,21 b	1,085 bc	80,18 c	0,0303 g	55,19 cd	59,02	37,21	26,46	5,23	5,10	4,32	0,63	12,85
8,8 ai + R	1073,44 cd	0,429 d	88,76 bc	0,0326 e	54,17 d	57,98	37,12	25,74	5,63	5,76	4,44	0,70	13,65
Degree of Significance	**	**	*	**	**	NS	NS	NS	NS	NS	NS	NS	NS

**Values followed by different letters in a column were significantly different ($p \leq 0.01$) using Duncan's multiple range test. NS: not significant.

Figure 1: Flower damage after8.8 ai tembotrione treatment.

Figure 2: Flower damage after2.2 ai tembotrione treatment**.**

Statistically significant differences were found between applications in terms of average fruit weight (g). The highest average fruit weight was obtained in 0 ai + R application, while the lowest was obtained in 4.4 ai + R application. In the 2.2 ai + R application, a 6.8% decrease was observed in the average fruit weight compared to the 2.2 ai application, while a 18.4% decrease was observed in the 4.4 ai + R application compared to the 4.4 ai application. In the + R application, this decrease increased to 31.8% compared to the 8.8 ai application. The authors thought that these values of average fruit weight in herbicide-only applications might be result of tembotrione causing flower deformation on tomato plant and therefore decreased the fruit set. While the average fruit weight increased as the tembotrione doses increased, it was observed that the average fruit weight was closer to each other in tembotrione + R applications. The reason for this is thought to be the gibberellin used in recovery application. De Jong et. al. (2009) reported that good fruit set depends on successful pollination and fertilization, which triggers fruit development with the activation of auxin and gibberellin. In addition, it has been reported that 60-90 ppm GA_3 applications during flowering or fruit set stage where plant growth regulators such as auxin and gibberellins affected fruit set rate and parthenocarpic fruit formation, reduced fruit set by 38-40% and increased fruit yield by more than 50% (Çömlekçioğlu & Şimşek, 2014)

Relative growth rate (RGR), found statistically significant between the applications. Results show that Control (0 ai) and Control + R treatment gave the highest dry matter production in a day. This result may be a proof of the negative effect of tembotrione when drifted on the plant. Data also shows that daily vegetative dry matter production is higher in plants treated with R. Moreover, RGR affected negatively with the increased treatments of tembotrione doses.Fruit diamater (mm), Hue°, Croma, SSC % EC, pH, TA % and vitamin C were found statistically insignificant.

CONCLUSIONS

It was concluded that the herbicide with tembotrione active ingredient, drifting towards the tomato fields, decreased the yield in general. Besides flower deformation can occur as a result of drift on plants. An approach like recovering the plant by using plant growth regulators and extra fertilizers may solve the problem. Although the decreased yield was slightly increased with the R application; the desired yield could not be achieved at the dose of 8.8 ai and resulted in great economic losses for the producer. Farmers often need treatments where plants are recovered to health quickly when their products are contaminated by tembotrione or another off-target herbicide. Therefore, the development of the R application studied in this research can be a source of inspiration for future studies. For instance, using other plant growth regulators for recovery formula may be a starting point. On the other hand, the recovery treatment was applied once in order to alleviate the negative effects of tembotrione in recent study. It is also a matter of curiosity how a scientific study in which the recovery treatment is repeated will contribute to the reduction of the effect of tembotrione.

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CHAPTER 6

THE EFFECTS OF THYME, ROSEMARY AND BASIL ON PHYSICO-CHEMICAL PROPERTIES AND VOLATILE COMPOUNDS IN EXTRA VIRGIN OLIVE OIL

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INTRODUCTION

Today, consumers have turned their attention to functional foods due to increasing health problems and improved quality of life. Table olives, olive-based products, antioxidants, phenolic compounds, anthocyanins, and other bioactive compounds (oleic acid, omega-3, omega-6) are included in the definition of "*nutraceutical substances*" and have a health-protective effect (trying to lower the risk of developing coronary artery disease, acting as anticarcinogens, and reducing inflammation) as well as a significant role in nutrition (Tokuşoğlu, 2008). The growing consumer demand for olive oil and olives (Rallo et al., 2013), contributes to an increase in the diversity and thus the economic value of these products. Flavored olive oils (FOO) were introduced in this context to increase the added value of olive oil and to attract consumers outside of the Mediterranean region to olive oil (Akçar and Gümüşkesen, 2012).

Around the world, extra virgin olive oil (EVOO) is widely regarded for its nutritional and olfactory qualities. Phenols are one of the many substances in the oil that stand out for providing the bitter-pungent flavor as well as for having antioxidant characteristics that aid to safeguard human health (Díaz-Montaña et al., 2022).

Secondary metabolites (alkaloids, terpenoids and phenolics) found in medical and aromatic plants contain important bioactive substances in order for people to live healthier and better-quality lives (Fig.1). Several studies have been carried out to determine which species are suitable for use. In accordance with the results, the use of those observed useful for both food and treatment purposes continues (Kırıcı et al., 2020).

Fig. 1. Important aromatic medicinal plants rich in essential oil components (URL-1).

The demand for medicinal and aromatic plants in world markets is increasing day by day. Many herbs, which are in the spice or condiment class, also carry medicinal and aromatic properties and are used in many areas such as medicine, perfume, cosmetics, hygiene products, soft drinks, beverages, confectionery, bubble gum, tea, essence, aroma (Bayram et al., 2010). One of the areas where highly aromatic plants are used is the production of flavored olive oil. According to Pişkin (2007), the most important family rich in aroma and essential oil components is the *Lamiceae* family, which includes rosemary, basil, thyme and mint (Güler, 2019).

The therapeutic and aromatic properties of plants are being added to olive oil, improving its sensory properties. Olive oil's oxidative stability is altered by the addition of plants and substances with antioxidant and prooxidant qualities, which has an impact on the oil's quality and shelf life (Gülkun, 2021).

When the research on flavored olive oils is examined, it is seen that the use of rosemary, basil and thyme is quite prominent. Türkiye's flora is very rich in these species, and they are cultivated in our country for both collection and production. Rosemary, basil, and thyme have significant potential in the use, dissemination, and sustainability of flavored olive oils produced in our country and in the future, increasing market share and converting them into economic income.

Türkiye is considered one of the most important countries in the world for the production and export of thyme plants. It has numerous applications, particularly in the pharmaceutical, cosmetic, and chemical industries, and is derived from natural flora and cultured (URL 2). Terpineol, linalol, carvacrol, cymol, and thymol, as well as p-simen and borneol, are present in the herb's volatile oil. Thymol and carvacrol, which give the plant its scent, are the primary components of thyme volatile oil. Thymol is present in higher concentrations in the essential oil of the Thymus type thyme, while carvacrol is primarily present in the essential oils of Thymbra, Origanum, and Satureja thyme. Thymol is a potent antibacterial that, in some nations, can be used as the only flavoring agent in food. Carvacrol increases shelf life by preventing food deterioration through its antibacterial and antifungal properties (especially the aspergillus mushrooms that produce aphlatoxin). Turkish thyme and thyme oil are rich in carvacrol. While it prevents food from becoming rancid and spoiled through its antioxidant and antimicrobial effects in food storage and extending shelf life, it also does not carry the negative health effects associated with additives (URL-2; Gülkun, 2021).

Loamy-clay alluvial soils and temperate climates greatly promote thyme's development. The plant can be grown in areas with features of a continental climate because it is tolerant of cold and drought. Thyme is a plant that can be cultivated in irrigated and dry conditions. In dry agricultural areas, the yield is around 125 kg/da, and it increases about 3 times under irrigation conditions. In thyme agriculture, the oil rate is high in dry conditions and the yield is low, while the oil yield is low in irrigated conditions (URL-2)

Thyme cultivation (Fig.2.) in our country is mostly done from seedlings, and both seeds and cuttings can be used as production material. Seed production is preferred because it is practical and less costly, but thyme seeds cannot be planted directly in the field because they are very small. Seedlings from seedbeds or viols are sown and transplanted to the field. Considering the climatic conditions, seedlings in seedbeds prepared in late autumn or winter are ready for planting after approximately 3-4 months (URL-2).

Fig. 2. Cultivation of thyme in field conditions (URL-3)

Rosemary, which is native to regions where the Mediterranean climate prevails, is an important plant whose essential oils, spices and extracts are used. Rosemary production in our country is large most of them are collected from nature on the Mediterranean and Aegean coasts. In Izmir, Antalya, Muğla and Afyonkarahisar provinces, agriculture has started in small and medium-sized areas (URL-4).

In the world, two types of rosemary oil are commercially available. These are the Spanish and Tunisian-Moroccan varieties. According to the essential oil analysis results, rosemary essential oil contains between 50 and 98 volatile components. According to the European pharmacopoeia, Spanish type rosemary essential oil includes 1.8-sineol (15-25%), camphor (13-18.5%), α-pinene (18-26%), camphene (8-12%), β-pinene, myrcene, limonene, bornyl acetate, borneol, and verbenone, whereas 1.8-sineol (38-55%), camphor (5-15%), α-pinene (9-14%), camphene (2.5-6.0%), β-pinen (4-9%), bornyl acetate, borneol, verbenon, and linalool are present in Tunisian-Moroccan type rosemary oil (Tisserand and Young, 2014; URL-4).

The leaves, volatile oil, and other byproducts of the rosemary plant are still utilized today (URL-4). Studies have revealed a rise in the use of aromatic and medicinal herbs as food preservatives in recent years. In 2008, the European Food Safety Authority (EFSA) approved the use of rosemary extras as preservatives, and rosemary extract with the code E392 was added to the European Union Food Additives legislation (URL-5).

The collection of rosemary is made from forest areas as a non-wood forest products and from field edges in the area of spread, and is now produced in a small amount of field conditions (Fig.3) (URL-4).

Fig. 3. Cultivation of rosemary in field conditions (URL-6)

Rosemary production usually starts in mid-July and continues in August-September. The most common method of production is vegetative production, which includes bare-rooted seedlings, rootless cuttings, and tube seedlings. The time required to prepare seedlings is determined by the field planting and harvesting schedule, and the seedlings must be grown in the nursery for at least 6 months (URL-4).

Basil is a spice as well as an ornamental plant. The genus *Ocimum* of the *Lamiaceae* family contains between 50 and 150 grassy and bushy species, has a very strong medicinal effect as an antimicrobial, and contains a high amount of vitamins and minerals (Karık et. al., 2014). Purple-colored varieties are common in some eastern provinces, including Western and Southern Anatolia, and the plant is known as reyhan in these areas. The green-colored varieties found in the western provinces are also known as basil (*Ocimum basilicum* L.) plants (Akalan, 2018; Dumanoğlu and Mokhtarzadeh, 2021). In addition to sowing the seeds directly into the field (URL-8), it is also produced as seedlings due to climatic conditions. Since basil prefers nutrient-rich soils, it should be used additionally in chemical fertilizers (URL-7).

According to Hiltunen and Holm (1999), a high-quality basil oil contains 30% to 90% linalool and 50% to 90% methyl chavicol (estragol) (Akalan, 2018).

Fig. 4. Cultivation of basil under field conditions (URL-8)

The purpose of this review is to assess the techniques for incorporating thyme, rosemary, and basil plant extracts into olive oil, as well as the impact of these plant extracts on the physicochemical qualities and volatile components of olive oil.

1. PROCESSING OF FLAVORED OLIVE OIL

Turkish Food Codex defines "*Natural olive oil*" as a clear, green to yellow color, unique taste and odor obtained from the olive tree fruit in a thermal environment that will not change its natural qualities, only by applying mechanical or physical processes such as washing, leaking, centrifugation and filtration while defining it as an oil that can be consumed as food in its natural state. It defines "*Flavored olive oil"* as oils obtained by flavoring natural cooking olive oils by adding different spices, fruits and vegetables, or their natural flavorings, and having other features other than taste and smell, which have the characteristics given for natural olive oil (Fig.5). FOO is oil with free fatty acid in terms of oleic acid not more than 0.8 grams per 100 grams (TSE, 2010). While FOO cannot be considered as EVOO according to EU regulation 1989 (2003), there is no regulation in the USA.

Fig. 5. Flavored EVOO (URL-9).

Production of flavoured oil has long been a tradition in the Mediterranean region. In the first applications, in order to protect the oil from deterioration reactions and to flavor it, the vegetable materials as seasoning source were dipped into pressed olive oil and maceration was carried out. The flavored oils after maceration were used as a sauce for pasta, salad dressing and dip for bread. In the following periods, with the Mediterranean region becoming more involved in international trade, FOO has been recognized all over the world and this led to an increase in consumer demand as an alternative product for consumers unfamiliar with the taste and aroma of natural olive oil around the world, as it contains different flavors and has a wide range of uses (Antoun and Tsimidou, 1997; Baiano et. al., 2010).

The methods used for flavoring olive oils are:

1. Maceration with seasoning,
2. The addition of seasoning during the olive kneading process (malaxation),
3. The addition of essential oil to the oil.

Maceration is the oldest method used for flavoring olive oil. Herbs, spices, and fruits are mixed with oil and kept at room temperature for a certain period of time. In the malaxation method, the resultant mixture is filtered to get rid of turbidity and solids. The added herbs, spices and fruits are kneaded together with the olive paste and clear and safe FOO are obtained. Addition of essential oil to olive oil provides high reliability. Because many spices and plants carry spores produced by *Clostridium botulinum*, adding essential oils to olive oil instead of these herbs allows for greater production flexibility (Ayadi et al., 2009; Baiano et. al., 2010; Issaoui et al., 2011). The choice of method used in the flavoring and flavoring process is very important as it affects both the oxidation stability and sensory acceptability of the oil (Dalgıç et. al., 2016).

Generally, it is seen that the amount of natural flavoring substances and aromatic plant extracts to be added to olive oil is determined in accordance with consumer preferences by making sensory analysis.

When the literature is examined, it is seen that olive oil is flavored with many different herbs and plant extracts. Table 1 shows studies on olive oils flavored with thyme, rosemary, peppermint, lemon, citrus, *Artemisia herba alba*, *Thymus algeriensis*, hot pepper, gooseberry, green anise seeds.

Table 1. Studies on olive oils flavored with different aromatic plants using olive oil flavoring method (2011-2021).

Material (Dried)	Method	Amount of material added	Reference
Thyme, rosemary and peppermint	Classic maceration	2.5% (w/w)	Dıraman and Hışıl, 2010.
Lemon, thyme	Classic maceration	20, 40, 60 and 80 g/L	Issaoui et al., 2011
Natural flavoring agents (thyme, basil, citrus and rosemary) Aromatic plant extract (thyme, basil, hot pepper)	Malaxation process	Thyme (%0.05 mL/mL), basil, citrus and rosemary (%0.07 mL/mL) Thyme (%20 (mL/mL), basil and hot pepper %40 (mL/mL)	Akçar and Gümüşkesen, 2012
Artemisia herba alba, *Thymus algeriensis*	Classic maceration	2.5, 5 and 7.5% (w/w)	Zouari et al., 2013
Red hot chili pepper	Classic maceration	10 and 20% (w/w)	Caporaso et al., 2013
Gooseberry	Malaxation process	0.40-1.98%	Dalgıç et. al., 2016
Rosemary	Malaxation process	0.41-2.00%	Yılmazer et al., 2016
Green anise seeds	1.Classic maceration 2.Ultrasonic assisted maceration 3. Direct addition of the essential oil	1. 15% (w/w) 2. 15% (w/w) 3. 0.33 mL	Moustakime et. al., 2021

2. THE EFFECT OF THE ADDITION OF THYME, ROSEMARY AND BASIL ON THE PHYSICO-CHEMICAL PROPERTIES OF OLIVE OILS

In a study in which 2.5% by weight dried thyme, rosemary and mint were added directly to the samples of virgin olive oil, refined hazelnut and edible vegetable oil without any treatment, it was found that the samples of rosemary and mint added natural olive oil had a slightly higher peroxide level than the control sample (Dıraman and Hışıl, 2010). A one-week heat treatment and air, rosemary and mint were reported to increase oxidative stability. In addition, in this study, it was reported that the direct addition of some spices (thyme and rosemary) to vegetable oils increased their shelf life and did not have a negative effect on fatty acid compositions.

Issaoui et al. (2011) researched oxidative evolution of virgin and lemon and thyme extracts FOO under thermo-oxidation processes. It was reported that no significant difference was observed between the oxidative stability of flavored and non-flavored oils.

The FOO prepared with thyme, basil, rosemary and citrus flavors, the EVOOs used in the preparation of these oils, the FOO prepared with the natural flavors supplied from the market (thyme, basil, rosemary, citrus and some garlic FOO prepared using the aromatic extras of olives and garlic herbs) was researched by Akçar and Gümüşkesen (2012). In this study, it was stated that the addition of rosemary aroma produced the highest increase in the induction period. It has been reported that the induction periods of commercially available samples are generally higher. In addition, it has been stated that the shelf life of FOO prepared using natural aromas varies between 9-16 months, the shelf life of olive oils prepared with aromatic plant extracts varies between 8-13 months, and the shelf life of FOO obtained from the market varies between 14-19 months.

In a study investigating how olive oil flavored with thyme, oregano, a mix of herbs (used as pizza seasoning), rosemary, and basil effects consumer preference, it was stated that herbs added to olive oil didn't cause significant differences in quality parameters and oxidative stability. However, it was stated that olive oil with thyme added slightly ahead of other oils in preventing oxidation (Issaoui et al., 2016). Additionally, it has been reported that the chlorophyll values of the flavored oils vary between 8.1-9.2 and the carotenoid values in the range of 4.9-5.2. It was stated that there was no significant effect on the chlorophyll and carotenoid values of these oils compared to the control sample (Chemlali EVOO), and only a slight increase in the chlorophyll values of basil flavored oil.

In a recent research investigating the effect of ultrasound technology, on the aromatization process of olive oil with rosemary and basil both by ultrasound (Soares et al., 2020), it was reported that rosemary FOO improved the stability and the induction time, while in the meantime presented low values for the quality parameters. In addition, it was reported that basil FOO significantly increased the quality parameters values, while it decreased the total phenolic compounds, antioxidant capacity and induction and stability times. Ultrasound-assisted maceration with basil achieved better results in just 10 minutes compared to conventional maceration. In addition, it was emphasized in the study that the use of basil would be a suitable choice for this method.

3. THE EFFECT OF THE ADDITION OF THYME, ROSEMARY AND BASIL ON THE VOLATILE COMPOUNDS OF OLIVE OILS

Issaoui et al. (2011) examined oxidative evolution of virgin and lemon and thyme extracts FOO under thermo-oxidation processes. It was reported that the highest percentage of carvacrol (37.6%) was detected in FOO treated with 80 g kg-1 of thyme.

In Issaoui et al. (2016)’s study, the volatile component properties of olive oil flavored with thyme, oregano, a mix of herbs (used as pizza seasoning), rosemary, and basil were investigated and it was stated that the transfer of aromatic compounds to EVOO depends on the type of aroma profile of the added plant. However, it has been noted that some flavors (rosemary, thyme, thyme and basil) have strong flavoring properties, while the herb mix used for pizza affects the flavor less and allows the perception of typical flavors in EVOO. About 27 compounds have been identified in oregano FOO and it was stated that the main components are 1,8-cineol (36.1%), p-cymene (15.6%), α-pinene (6.9%) and β-pinene (6.3%). In general, it was stated that aldehydes predominate in EVOO and terpenoids in FOO.

In another study, aroma transition from rosemary leaves during aromatization of olive oil was studied (Yılmazer et al., 2016). It has been stated that when the aromatic material is mixed with olive paste at the malaxation stage or during infusion compared to the crushing stage, the aroma compounds are transferred from rosemary to olive oil without being affected by malaxation conditions.

According to a recent study (Soares et al., 2020), using ultrasound technology instead of traditional maceration to aromatize olive oil with rosemary and basil resulted in a considerable increase in the amount of total phenolic compounds, terpenes, esters and ketones present.

CONCLUSION

Adding thyme, rosemary and basil to olive oil, which has a strong aroma, offers the consumer an alternative taste. In addition to offering a different flavor, the addition of these plants to olive oil does not adversely affect the quality of olive oil. At the same time, it has been concluded that it increases oxidative stability and shelf life in studies. Additionally, prior studies have revealed that the volatile compounds found in thyme and basil are easily transferred to olive oil. In addition to the thyme, rosemary and basil FOO investigated in this review, offering different plant extract additions to the consumer will play a role in increasing the product range.

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CHAPTER 7

IMPROVEMENT OF AGRICULTURE BY MICROORGANISMS

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1. INTRODUCTION

The requirements of plants for microorganisms are much more than you can imagine. The interaction that starts from seed germination, seedling, or sapling production continues during the cultivation of the crop, harvest, storage, and even consumption. The ancient relationship between plants and microorganisms, which has developed and matured with evolution, turned into loyalty. Some abilities of microorganisms are indispensable in the growth and development of plants.

The fact that the soil has a rich structure that contains both plants and microorganisms creates an environment for a symbiotic relationship in the plant root zone. The biofertilizer ability of microorganisms is very beneficial to the life of plants. While they convert the nutrients into a form suitable for the plants' uptake, they also perform the fixation of atmospheric nitrogen. They contribute to plant growth and development with the growth regulators they produce. Microorganisms surrounding the root environment create a tampon zone between the plant and pathogens, consume the nutrients of pathogens with their antagonistic properties, and prevent the development of pathogens with antimicrobial substances. Produced metabolites act as vaccines, stimulating the immunity of plants, and increasing their resistance to pathogens, physiological stress, and environmental stress.

Biofertilizers and biopesticides produced with microorganisms support sustainable agriculture systems with environmentally friendly production methods.

In this article, we will try to give information about two of the most important ways of using microorganisms in agriculture: Biofertilizers and biopesticides. The microorganisms mentioned here are the most commonly used ones in agriculture.

2. BIOFERTILIZERS

Biofertilizers improve the availability of nutrients such as phosphate, zinc, and silica in the soil for the plant by increasing the number and activity of beneficial microorganisms in the root zone. Interactions occur between microorganisms, plants, and the soil in the rhizosphere. Communication between plants and soil microorganisms continues at the biochemical level through various molecules (Pinton et al., 2000). Some of the bacteria in the rhizosphere are beneficial for plants, some are neutral, and some are pathogenic. 2-5% of the bacteria in the rhizosphere are 'plant growth-promoting rhizobacteria' (PGPR) (Kloepper and Schroth, 1980). The number of bacteria around the root is much higher than in the rest of the soil. Thus, they benefit from the substances secreted from the plant roots (Carvalhais et al., 2015).

Rhizobacteria have emerged from numerous studies that support plant growth in different ways. The bacteria support plant growth by biological nitrogen fixation, plant growth regulator/phytohormone production, phosphate, zinc, and silica dissolution and mobilization, increasing mineral and water uptake, promoting root growth, and increasing enzyme activity in the plant (Ferreira et al., 1987). In addition, rhizobacteria perform detoxification of heavy metals (Wani and Khan, 2010), degradation of herbicides (Ahemad et al., 2012), salinity tolerance (Mayak et al., 2004), biological control of plant diseases and pests (Hynes et al., 2008).

Biological nitrogen fixation refers to a process carried out by microorganisms based on the enzymatic conversion of atmospheric nitrogen to ammonium, which can be easily absorbed by roots. Biological nitrogen fixation accounts for about two-thirds of fixed nitrogen globally (Gouda et al., 2018). Biological fixation of atmospheric nitrogen takes place with the help of microorganisms such as *Rhizobium*, *Clostridium*, *Azobacter*, *Bacillus*, and *Klebsiella* (Shridhar, 2012). Symbiotic rhizobacteria transform structural and physiological modifications of bacterial cells and plant roots into specialized structures called nodules. Other nitrogen-fixing bacteria are free-living organisms common in cultivated fields (Aasfar et al., 2021). A study conducted in Australia with an intensive wheat rotation farming system showed that free-living microorganisms contribute 20 kg per hectare per year (30-50% of the total requirement) to the long-term nitrogen requirements of this crop-growing system (Vadakattu and Paterson, 2006).

Phytohormones produced by microorganisms improve the host's tolerance to abiotic stresses and stimulate plant growth. The effects of phytohormones produced by microorganisms such as auxins (Etesami et al., 2015; Pereira et al., 2016), gibberellins (Khan et al., 2014), cytokinins (Kudoyarova et al., 2014) and ABA (Sgroy et al., 2009) in plants were similar to those of exogenous phytohormones (Egamberdieva, 2009; Turan et al., 2014; Shahzad et al., 2016).

Today, many rhizobacteria (*Rhizobium, Sinorhizobium, Gluconacetobacter*, *Azorhizobium*, *Stenotrophomonas*, *Mesorhizobium*, *Bradyrhizobium*, *Agrobacterium*, *Ochrobacter*, *Arthrobacter*, *Bacillus*, *Pseudomonas*, *Acetobacter*, *Azospirillum*, *Pantoae*, *Azotobacter*, *Beijerinckia*, *Zoogloe*, *Arthrobacter*, *Derxia*, *Allorhizobium*, *Enterobacter*, *Alcaligenes*, *Herbaspirillum*, *Acinetobacter*, *Klebsiella*, *Azoarcus*, *Rhodococcus*, *Serratia*, *Burkholderia*) are commercially produced as either biocontrol agents or biofertilizers (Çakmakçı, 2005; Gray and Smith, 2005; Babalola, 2010; Disi et al., 2019).

Information about the most commonly used microorganisms as biofertilizers is given below.

2.1. *Pseudomonas* spp.

Pseudomonas spp. are fully catalase-positive, gram-negative, rod-shaped, multi-flagellate bacteria from the *Pseudomonadaceae* family. Some strains of these bacteria fluoresce under UV light. Usually found in water and soil, *Pseudomonas* is the most populous genus among gram-negative bacteria species (Gomila et al., 2015).

Pseudomonas, which can be grown rapidly and mass-produced in vitro, has plant growth promoting and biocontrol properties as a rhizobacterium. The bacteria can colonize and reproduce quickly in the rhizosphere because they can use seeds and root secretions quickly. *Pseudomonas* spp. can produce many antibiotics, siderophores, and plant growth-promoting substances in the rhizosphere of such plants, which are highly adaptable to environmental stresses and aggressively compete with other microorganisms. *Pseudomonas* induces systemic resistance in the plant and suppresses the growth of pathogens, has a highly developed ability to fix nitrogen and dissolve phosphate compounds (Baliah et al., 2018).

Inoculation of *Pseudomonas* isolate on pea seedling (*Rhizobium leguminosarum*) increased nodulation by 156.2%, plant biomass by 57%, chlorophyll content in nodules by 31.5%, iron content by 95.9%, and leghemoglobin concentration by 17.5 times. In addition, the nitrogen uptake of the shoots was 66.3%, the phosphorus uptake was 23.3%, the potassium uptake was 47.1% and the zinc uptake was 2.75 times higher (Mishra et al., 2012).

Mung bean (*Vigna radiata*) was inoculated with *Pseudomonas fluorescens* bacteria and it was determined that there was a 15% increase in root length, 4.7 times in root nodulation, 7 times in fresh nodule weight and 21 times in dry nodule weight compared to control (Shaharoona et al., 2006).

The effect of *Pseudomonas putida* on drought stress in two different chickpea (*Cicer arietinum* L.) cultivars was studied, and bacterial inoculation significantly increased the germination rate. Growth parameters, plant water content, membrane integrity, osmolyte accumulation, reactive oxygen species excretion ability and the activity of stress sensitive genes were negatively affected in plants under stress, but bacterial application had a positive effect on these properties (Tiwari et al., 2006).

2.2. *Bacillus* spp.

Bacillus species that are a significant component of the soil microflora are aerobic and facultative anaerobic, gram-positive endospore bacteria. *Bacillus* spp. Are mainly safe microorganisms and can synthesize many substances used for agricultural and industrial purposes (Logan and Rodriguez-Diaz, 2006).

Some *Bacillus* species are a group of plant growth-promoting rhizobacteria that have direct and indirect mechanisms. While they help in nitrogen fixation, dissolution and mineralization of phosphorus, stimulation of arbuscular mycorrhizal fungi growth, triggering of induced systemic resistance, and development of tolerance to biotic and abiotic stresses, they also synthesize siderophores (iron chelating compounds), antimicrobial compounds, phytohormones and hydrolytic enzymes (Goswami et al., 2016).

In cases such as lack of water in the soil, salinity problem, and heavy metal toxicity, they produce polysaccharides and siderophores that prevent the movement of toxic ions, and regulate the ionic balance and water transport in plant tissues. The polysaccharides secreted into the environment by microorganisms are natural polymers with high molecular weight, recyclable, and environmentally friendly. With stress, their production increases, and they protect microorganisms against toxic compounds and osmosis. In addition, *Bacillus* regulates the intracellular phytohormone metabolism, synthesis of indole-3-acetic acid, gibberellic acid, and 1-aminocyclopropane-1-carboxylate deaminase and increases the plant's tolerance to stress (Radhakrishnan et al., 2017).

2.3. *Azotobacter* spp.

Azotobacter species, a PGPR group, are free-living, non-endospore-forming, gram-negative, aerobic, non-symbiotic, oval or spherical-shaped, thick-walled microcysts and N-fixing bacteria that commonly live in soil, water, and sediments. Different factors such as pH, phosphorus content, soil aeration, and moisture content affect *Azotobacter* population in the soil. *Azotobacter* spp. can grow and survive in extreme environmental conditions, even in dry soils with high salt concentrations, pH values, and even maximum temperatures. *Azotobacter chroococcum* can survive at 45°C, up to pH 8 and 6% NaCl concentration (Mirzakhani et al., 2009; Akhter et al., 2012).

Azotobacteria are known to produce various vitamins, amino acids, plant growth hormones, antifungal agents, hydrogen cyanide, and siderophores. Indolacetic acid, gibberellic acid, arginine, etc. produced by *Azotobacter*. Growth-promoting agents, such as growth-promoting substances, have a direct effect on the shoot and root length and seed germination of various crops (Barazani and Friedman, 1999; Garg et al., 2001; Ahmad et al., 2008). *Azotobacter* species inhibit the growth of common plant pathogens, *Fusarium*, *Aspergillus*, *Alternaria*, and *Rhizoctonia* species (Agarwal and Singh, 2002; Cavaglieri et al., 2005; Bhosale et al., 2013).

The increase in the production of nitrogen and plant growth regulators stimulates leaf, root, branching, flowering, and fruit development, dry matter accumulation, and leaf area increase in *Azotobacter* inoculated plants. Mangrove (*Rhizophora apiculata*) seedlings were inoculated with *Azotobacteria*, root biomass increased by 98.2%, root length by 48.45%, leaf area by 277.86%, shoot biomass by 29.49%, chlorophyll amount by 151.0% and carotenoid amount by 158.73% compared to controls (Ravikumara et al., 2004). Yield increased by 10-12% and leaf area by 3.5% in canola plants inoculated with *Azotobacter*. When the *Azotobacter* application was continued, the yield according to the chemical fertilizer increased by 21.17% (Yasari and Patwardhan, 2007).

2.4. Mycorrhizal fungi

Mycorrhiza defines as a mutually beneficial relationship between the root of a plant and a fungus that colonizes the plant root. In many plants, mycorrhiza are fungi that grow inside the plant's roots, or on the surfaces of the roots. Mycorrhizal fungi form a mutual symbiosis with plants and infect roots without causing root disease. The fungi can be found in the rhizosphere of most plants and form associations with all gymnosperms and with more than 83% of dicots and 79% of monocots. Fungal hyphae allow the roots to come into contact with a larger volume of soil (Kennedy and de Luna, 2005).

Mycorrhizal fungi collect mineral nutrients such as nitrogen and phosphorus from the soil and transport them to the plant rhizosphere (Fitter 2005), support plant growth and root system development by interfering with the phytohormone balance of host plants, and provide tolerance to soil and environmental stresses (Gutjahr and Paszkowski, 2013; Rouphael et al., 2015). In addition, mycorrhizal fungi also increase the resistance of plants to diseases and pathogenic organisms (Nagy et al., 2009).

Numerous studies have shown that mycorrhizal fungus can improve plant health and yield. A large part of horticultural production involves sterile micropropagation in vitro. A critical developmental point of plantlets produced in this way is transferred to soil, which can cause losses. Inoculation of micro-grown fruit trees with mycorrhizal fungus during transplant improves growth and nutrient uptake, resulting in larger plants and improved commercial properties (Lovato et al., 1992; Cordier et al., 1996; Schubert and Lubraco, 2000). The mycorrhizal fungus can accelerate this transition and improve the health of plantlets. In addition, the application of mycorrhizal fungi can allow for a reduction in the amount of fertilization without reducing the yield, thus increasing profitability (Vestberg et al., 2002).

A field trial was conducted with *Rhizophagus irregularis* for potato production in 231 fields in Europe and North America. It showed a significant increase in tuber production after grafting. As a result of the experiment, the average yield increase was obtained as 3.9 tons/ha (Hijri, 2016).

Glomus species are the most common genus among mycorrhizal fungi. Vine plants inoculated with *Glomus fasciculatum* had a remarkable increase in phosphate content (525.13%), total chlorophyll content (18.88%), leaf number (75.29%), leaf area (209.96%), shoot length (34.88%), and root length

(19.67%) compared to non-inoculated plants (Borde et al., 2009). A similar trial was conducted with *Glomus iranicum* on cuttings and grafted vines. In the experiment, a 106% increase in root volume in cuttings, 36% in grafted vines, and a 45% increase in root dry weight in cuttings and 30% in grafted vines were achieved (Luciani et al., 2019).

3. BIOPESTICIDES

Biopesticides can be defined as biological materials with formulations using microorganisms that damage agricultural pathogens and pests. Commercial preparations for the biological control of plant diseases rely on the practical application of rhizosphere-survivable bacterial and fungal species (Viterbo et al., 2002).

Chemical pesticides are responsible for the intense pollution of the environment. A serious health hazard occurs due to the presence of residues in food. They lead to loss of biodiversity and the development of resistance in targeted pest populations, and epidemics of secondary pests that cannot be controlled by their natural enemies, which are destroyed by chemical pesticides (Kaya and Vega, 2012). In the process of managing target pests, synthetic pesticides kill non-target beneficial organisms such as pollinators, predators, and antagonists thereby disrupting biodiversity (Ndakidemi et al., 2016).

Different regulations have been developed to limit the use of pesticides in agriculture, with the effect of awareness in the community about the harm of pesticides to the environment. The regulations emphasize the need for food safety and safe agriculture, and studies have been conducted on natural pesticides derived from microorganisms and plants (Budzinski and Couderchet, 2018; Dayan et al., 2009; Lorsbach et al., 2019). In addition, prohibitive restrictions are constantly being applied to reduce the number of pesticides with time. For example, while the active content of conventional pesticides was reduced from more than 1000 in 2001 to 250 in 2009, market penetration of new conventional pesticides decreased from 70 in 2000 to 28 in 2012 (McDougall, 2013).

Although biopesticides have been used in agriculture for a long time, extensive research activities on microbial biopesticides have only started as a result of successful practical applications of microbial species such as *Bacillus thuringiensis* and *Trichoderma harzianum*. With these studies, many new microbial species/strains and toxins have been discovered and their agricultural applications have been developed (Ruiu, 2018).

The use of biopesticides is, more advantageous than the use of their counterparts, traditional chemical pesticides. Unlike pesticides, microbial pesticides are eco-friendly, leave no harmful residues, and are often specific to the targeted pests. Normally the target pest and closely related organisms are affected, while the impact on non-target species is significantly less. The amount of water used in the production processes of microbial pesticides is much lower than pesticides. Biopesticides exert their inhibitory effects through multiple modalities of action, such as growth regulators, intestinal disruptors, metabolic poison, neuromuscular toxins, and non-specific multisite inhibitors. The methods, which can vary against the targeted pests, eliminate the chance of developing resistance, unlike pesticides (Sparks and Nauen, 2015; Dar et al., 2021).

The number of antagonists inoculated into the soil and the amount of nutrients in the soil are critical to the effects of the antagonists. Microbial pesticides and pathogens have similar growing conditions. Before the conditions in which pathogens will reproduce, the number of antagonists to be applied should be sufficient to spread over the entire target area. Application of organic soil improvers to the rhizosphere increases the effectiveness of microbial antagonists against pathogens. The ability of antagonists to produce antimicrobial metabolites, phytohormones, and immune system promoters is a factor of influence in the prevention of pathogens (Fenibo et al., 2021).

To increase the use of microbial pesticides, improvements should be made in the following areas: Optimizing the production processes of microorganisms, thus ensuring efficient production processes and reducing costs. Optimizing the formulation of microbial pesticides, which has a direct impact on the success and shelf life of the field applications of biopesticides, ensures that the products remain stable during storage and successful field applications. Microbial pesticides should be applied at the right time and rate against pathogens. For this reason, practitioners, especially farmers, should receive good training on these issues.

Information about the most commonly used microorganisms as biopesiticides is given below.

3.1. *Bacillus thuringiensis*

Bacillus thuringiensis is a gram-positive, spore-forming, rod-shaped soil bacterium. The defining feature of *B. thuringiensis* is its ability to produce toxic protein crystals, a bioinsecticide, during sporulation. *B. thuringiensis* strains produce different types of toxins, each affecting a different taxonomic insect. Completely biodegradable and harmless to humans, vertebrates, and plants, these proteins are specific for targeted insects. Therefore, it is a suitable alternative for the control of human disease vectors and crop pests. The toxin protein crystals produced during the sporulation stage are mainly composed of crystal (Cry) and cytolytic (Cyt) proteins, also called δ-endotoxins. Cry proteins have an insecticidal effect against *Coleoptera*, *Diptera*, *Hymenoptera*, and *Lepidoptera* insect~~s~~ orders. The efficiency of cry toxins on nematodes has also been described (Leyns et al., 1995; Wei et al., 2003; Baghaee Ravari and Mahdikhani Moghaddam, 2015; Yu et al., 2015; Huang et al., 2018; Liang et al., 2022). Cyt protein crystal with hemolytic activity is generally effective against the *Diptera*. Cyt toxins affect insect midgut cells and, together with Cry toxins, increase insecticidal damage. An important detail is that Cyt toxins overcome mosquitoes' resistance to Cry toxins (Soberon et al., 2013). The genes encoding the Cry/Cyt proteins become active in sporulation. RNA polymerase, a specific enzyme that becomes active during sporulation, is responsible for controlling these genes. Cry/Cyt toxins constitute 20% of the protein content of spores (Aronson, 2002).

After the operational techniques and formulations of *B. thuringiensis* have determined in the mid-1980s, it was used cumulatively in approximately 8.5 million hectares of forest in Canada, 3 million hectares in the United States, and 1.7 million hectares in Europe until 2000 for the control of deciduous trees such as the spruce budworm *Choristoneura fumiferana* (Frankenhuyzen, 2000).

3.2. *Trichoderma* spp.

Fungal-based biological control agents have found a wide range of use with bacteria due to their broad spectrum of disease control and production efficiency. The use of antagonist fungi in the fight against fungal plant diseases in biological control is of great importance (Demirci et al., 2002).

Trichoderma species are well-studied model fungal organisms used for their biocontrol properties with great potential to alleviate the use of agrochemicals in agriculture (Kubicek et al., 2019). Around 90% of fungal biocontrol agents against pathogenic microorganisms belong to different strains of *Trichoderma* (Hermosa et al., 2012). *Trichoderma* spp. are asexual, spore-producing, fungicolous ascomycete fungi, and the most frequently isolated soil fungus from plant root ecosystems and are particularly an antagonist and parasite of many plant pathogenic fungi.

By colonizing the soil or plant roots, *Trichoderma* physically takes over the area, creates a barrier to pathogens, and prevents the further spread of the pathogen (Witkowska and Maj, 2002).

Trichoderma spp. are saprophytic fungi that are known to stimulate systemic resistance in the plant, as well as increase the yield by promoting shoot and root development, increasing resistance to abiotic stress conditions, promoting promote nutrient uptake, and increasing improve photosynthesis (Verma et al., 2019; Adeleke and Babalola, 2021). , increasing plant growth and nutrient uptake, increasing the solubility of nutrients and minerals in the soil (Wilberforce et al., 2003), and promoting root growth.

The mechanisms of action of *Trichoderma* species include mycoparasitism (Yang, 2017), antibiosis (Zeilinger et al., 2016), competition (Benítez et al., 2004), stimulation of the plant defense system (Pieterse et al., 2014). Antibiosis is an important feature in deciding the saprophytic ability of the fungus. Antibiotics such as trichodermin, suzukacillin, and alameticin produced by Trichoderma harzianum affect phytopathogens morphologically or physiologically by successful penetration (Manibhusanrao et al., 1989). It has been reported to inhibit the growth of soil-borne fungi such as *Sclerotium* and *Verticillium* genera (Ragab et al., 2015). In addition, volatile and non-volatile antibiotic productions of twelve *Trichoderma* isolates were controlled in the experiment performed on *Phaseolus vulgaris* roots. All isolates inhibited mycelial growth of *R. solani*, which causes root and throat rot in plants (Bhagat and Pan, 2010).

Hydrolytic enzymes such as glucanase, chitinase, and protease are key players that confer mycoparasitism properties on *Trichoderma* (Haran et al., 1996). A trypsin protease purified from *T. harzianum* CECT 2413 strain showed nematicidal effects by damaging the eggs of *Meloidogyne incognita*, a harmful nematode (Suarez et al., 2004). In the other study, chitinase and protease enzymes produced by *Trichoderma* species helped control the peanut pathogen, *Sclerotium rolfsii* (Parmar et al., 2015).

3.3. *Beauveria bassiana*

The number of identified entomopathogenic fungal species is over seven hundred (Roy et al., 2006). The fact that entomopathogenic fungi, which do not affect mammals, do not create resistance in pests, maintain their effects in nature for a long time, be effective against insects in all development stages, can be used with pesticides, and do not present significant problems in field applications. *Beauveria bassiana* is a prominent biocontrol agent among the identified entomopathogenic fungi species. *B. bassiana* fungus can infect over 700 hosts, including insects orders such as *Lepidoptera*, *Blattariae*, *Dermaptera*, *Isoptera*, *Thysanoptera*, *Hymenoptera*, *Siphonaptera*, *Neuroptera*, *Hemiptera*, *Diptera*, *Embioptera*, *Orthoptera*, *Coleoptera*, and *Mantodea* (Zimmermann, 2007). Apart from *B. thuringiensis*, *B. bassiana* is the most commonly used biopesticide that can be effectively transmitted (Baldiviezo et al., 2020).

Insect cuticle penetration is the first step of entomopathogenic fungi infection, which involves mechanical forces, cuticle-degrading enzymes (chitinase, lipase, protease, etc.), and hyphae-produced specific infection structures (Chelico and Khachatourians, 2008). Under appropriate conditions, the conidia germinate to form hyphae and then secrete various insecticidal toxins (Lewis et al., 2009; Wanchoo et al., 2009). *B. bassiana* toxins are secondary metabolites and small molecular compounds, such as beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, oxalic acid, calcium oxalate crystals, and many beauvericin analogs. Among these, mycelia-secreted beauvericin is one of the most important toxins (Molnár et al., 2010; Rohlfs and Churchill, 2011; Safavi, 2013).

The effects of *B. bassiana* on the eggs and larvae of Tomato leafminer (*Tuta absoluta*; *Lepidoptera*: *Gelechiidae*) were investigated, and it was reported that the egg stage was successful with a rate of 41.67% on the seventh day and 66.67% on the ninth day (İnanlı et al., 2012).

B. bassiana was inoculated to control *Cosmopolites sordidus*, the pest of the banana plant (*Musa* spp.), and damage to the immature stages of banana plants obtained from tissue culture was reduced by 50% (Akello, 2008).

The effect of *B. bassiana* was tested on the sweet potato borer (*Cylas formicarius*), which causes significant damage in sweet potato (*Ipomoea batatas* L.) production, and the highest mortality rate (91.67%) was reached on the sixth day (Saputro et al., 2019).

3.4. *Actinomycetes* spp.

Actinomycetes spp. are members of the *Actinomycetales* and gram-positive, spore-forming, filamentous, aerobic, or anaerobic bacteria with high guanine-cytosine content (68-78%) in their genomes. *Actinomycetes* are traditionally considered a transitional form between fungi and bacteria, growing with a combination of tip elongation and hyphae branching, attracting attention with their environmentally friendly properties and economic values. *Actinomycetes* have biofertilizer properties such as contributing to nitrogen fixation and phosphate solubilization, increasing the amount of mineral matter, biological buffering of soil, degradation of high molecular weight toxic compounds such as hydrocarbons, converting organic materials into suitable compounds for plant uptake, increasing soil fertility, and producing plant growth regulators (De Schrijver and De Mot, 1999; Jansen et al., 2014; Barka et al., 2015; Bhatti et al., 2017).

Bacterial endophytes are an important class of microorganisms that help the host thrive while using the nutrients provided by plant cells. Endophytic *actinomycetes* produce secondary metabolites that form beneficial relationships with their host by protecting plants from phytopathogens and stimulating plant growth (Singh and Dubey, 2018).

Studies have shown the existence of *actinomycetes* metabolites active against bacteria, fungi, viruses, nematodes, insects, and weeds (Silva et al., 2022). In addition, *actinomycetes* produce volatile organic compounds that play an important role in their bioactivity, acting as antimicrobial agents and even causing changes in gene expression of nearby microorganisms (Pacios-Michelena et al., 2021).

Streptomyces is the most common soil *actinomycetes*, and some studies suggest that this genus represents more than 95% of total *actinomycetes* found in soil (Yadav et al., 2018). *Streptomyces* is responsible for synthesizing approximately 60% of all antibiotics used in agriculture and horticulture and is best known for its antibiotic production. Species belonging to the genus

Streptomyces have been identified as potential biocontrol agents against both foliar and soil-borne fungal diseases caused by important agricultural pathogens such as *Rhizoctonia solani, Colletotrichum musae*, *Fusarium oxysporum*, *Botrytis cinerea*, *Alternaria alternata*, *Sclerotinia sclerotiorum* (Ulloa-Ogaz et al., 2015).

Streptomyces produces a varied amount of phenol, pyrrolizidine, hydrocarbons, esters, and acids compounds associated with fungus inhibition and cell membrane destruction (Qi et al., 2019). Salvianolic acid B metabolite produced by *Streptomyces* can damage mycelial cells and spores of phytopathogenic fungi such as *Alternaria, Fusarium*, and *Botrytis* (Sharma and Manhas, 2020), while another metabolite (6-amino-5-nitrosopyrimidine-2,4-diol) have the ability to inhibit mycelial growth of phytopathogenic fungi such as *R. solani* (7.5-65%), *A. alternata* (5.5-52.7%), *Aspergillus flavus* (8-30.7%), *F. oxysporum* (25-44%), *Sarocladium oryzae* (11-55.5%), and *S. sclerotiorum* (29.7–40.5%) (Shahid et al., 2021). *Streptomyces* can also control fungal diseases by secreting extracellular lytic enzymes such as chitinases that can hydrolyze chitin found in fungal cell walls. (Nayak et al., 2020).

Determining the lethal effect of *Streptomyces griseoviridis* against *Galleria mellonella* larvae, which cause significant damage in beekeeping, it was revealed that *actinomycetes* can produce metabolites with insecticidal effect (Augustyniuk-Kram et al., 2007). Polyketide pesticides such as avermectins, milbemycins, and spinosyns produced by actinomycetes have effectively protected plants against various insect orders (*Siphonaptera, Isoptera*, *Blattodea*, *Diptera*, *Hymenoptera*, *Lepidoptera*, *Phthiraptera*, *Coleoptera*, and *Thysanoptera*) that cause significant damage in agriculture. (Batiha et al., 2020; Li et al., 2021).

4. CONCULUSION

Microorganisms help increase yields in agricultural production and prevent damage caused by bacteria, fungi, nematodes, weeds and insects. Microorganisms highly affect soil quality, plant growth and agricultural productivity, Soil microbial biomass and rhizosphere microbiome are indicators for determining soil fertility.

The rich soil content around the root attracts microorganisms. While they use these nutrients in common with plants, they also mediate for plants to reach soil elements that they cannot absorb for different reasons. Fertilizer use can be saved as they enable plants to use the soil more efficiently.

While rhizobacteria provide better growth of plants with the help of phytohormones they produce, they also support the elimination of damage caused by biotic (bacteria, virus, fungus) and abiotic (heavy metal, drought, salinity, temperature) stress, and stimulate the immune system. They can provide a similar effect in plant organs other than the root zone.

Biopesticides and biofertilizers can prevent the development of pathogens with the help of antibiotic metabolites they produce. They also protect plants by creating a physical buffer zone between the root and phytopathogens, breaking the connection between them.

Using these features of microorganisms for environmentally friendly agricultural production and trying to imitate nature can make our work a little easier. When formulating a new biopesticide or biofertilizer, the presence of multiple species and the identification of more efficient new lines offer 'endless possibilities'. Intensive laboratory and field studies are needed to discover new productive strains. Subsequently, formulation optimization is required to reflect the good properties of these strains to biopesticides or biofertilizers.

If some critical properties of biopesticides and biofertilizers are developed, they will be more widely used in agriculture. Since formulations contain living material, more protection from environmental conditions is needed. Maintaining the stability of the product after production and during storage is one of the most critical points. Well-designed formulations and the cold chain should enable microorganisms to reach their point of application from their production without losing their effectiveness. Another effect of formulation optimization is that they are optimized to minimize the effects of ambient conditions (e.g. sunlight) during application.

Studies have shown that inoculation of plants with microorganisms and signaling compounds to ensure continuous microbe-plant communication can be an effective strategy to promote crop growth. In addition, it is imperative that these strategies continue to evolve against abiotic and biotic stresses, whose effects are thought to increase under climate change conditions. The fact that only these strategies will work in difficult conditions makes the efforts more valuable.

Each factor related to the efficiency of biopesticides and biofertilizers in agriculture should be studied intensively, taking into account the needs of nature and agricultural production. Efforts should be increased to find solutions that already exist in nature for environmentally friendly, organic and sustainable agriculture.

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CHAPTER 8

BIOLOGICAL CONTROL BY ENTOMOPATHOGENIC NEMATODES IN PEST MANAGEMENT

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INTRODUCTION

Biological control is used for defining the use of insects, microbial pathogens or entomopathogenic nematodes (EPNs) to control the population of many significant pests occurred in agricultural areas. Biological control has been accepted as a strong and one of the most significant methods for a safe environment and sustainable agriculture because it is a main part of integrated pest management (IPM).

EPNs are among one of the best biocontrol agents in management of various insects. These nematodes were discovered in the 1920s, and received an interest in the 1950s, and their commercialization started in the 1980s. Numerous researches have been carried out all around the world for obtaining EPNs that have potential in control of significant pests. These nematodes differ from other nematodes as their hosts are infected in a short time because of their mutualistic bacteria. Due to their successful biocontrol activities and having many advantages over pesticides, EPNs are increasingly used as a biological control agent (Kaya & Gaugler, 1993).

The key points for obtaining success with EPNs are to comprehend their life cycles and functions, to combine the correct nematode species/strains with the pest insect species, to apply EPNs during proper environmental conditions (like soil moisture, soil temperature, and sunlight) and to apply them only with compatible chemicals. Because EPNs are living organisms, they need careful handling to survive shipment and storage as well as proper environmental conditions for their survival in the soil after application.

EPNs efficacy and compatibility with other biocontrol agents and pesticides support their incorporation in IPM. Furthermore developments in mass production and formulation technology of EPNs and the discovery of many successful isolates have led to an increased commercial and scientific interest in these beneficial organisms.

1. ENTOMOPATHOGENIC NEMATODES

EPNs are colourless, segment-less, elongated, insect-parasitic roundworms with lengths varying between 0.4-1.1 mm (Figure 1). These are a group of soil-borne organisms that attack soil-dwelling insects that live in, on or near the soil surface.

Figure 1: Infective juvenile of *Steinernema carpocapsae* (Hoffmann and Frodsham, 1993)

EPNs belong to the two major families Steinernematidae and Heterorhabditidae from Phylum Nematoda (Kaya & Gaugler, 1993; Laznik et al., 2010). These beneficial nematodes which have drawn attention from all over the world because they have proven to be promising biocontrol agents of significant insects of cryptic habitats, foliar and soil (Askary, 2010; Askary & Abd-Elgawad, 2017; Gozel & Kasap, 2015).

These beneficial organisms have been known since about the 17th century (Nickle & Welch, 1984), but it was only in the 1930s that important care was given to these organisms in pest control programme. Recently EPNs are accepted to be the most effective biological control agents of soil and above-ground insects (Glazer & Navon, 1990; Garcia del Pino et al., 2013; Gozel & Gunes, 2013; Van Damme et al., 2016).

Many studies on EPNs have been carried out in temperate, tropical and subtropical areas, and scientists emphasized that they have a global distribution because they have been obtained from nearly all the continents. The only continent where EPNs have not been searched from is Antarctica (Hominick, 2002). Surveys have shown that host availability, soil temperature, and texture are major factors in determining the distribution of EPNs (Hominick & Briscoe, 1990; Stock et al., 1999). Until now, nearly 100 valid species of *Steinernema* and 21 species of *Heterorhabditis* have been recorded but surveys on these beneficial organisms still continues (Bhat et al., 2020).

1.1. Biology of Entomopathogenic Nematodes

Steinernema and *Heterorhabditis* are known by their symbiotic bacteria, *Xenorhabdus* and *Photorhabdus*, respectively and these bacteria are responsible for killing their hosts (Moazami, 2002) (Figure 2).

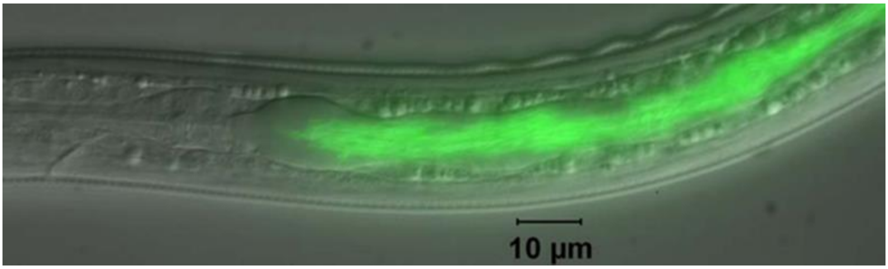

Figure 2: *Photorhabdus luminescens*, the symbiotic bacteria of *Heterorhabditis bacteriophora* (photo by Todd Ciche)

The 3rd juvenile stage of these nematodes is called as “infective juvenile” (IJ) or “dauer juvenile”. Infective juvenile is the only free-living stage, found outside of the insect that can able to target host and survive for a few months in soil until susceptible insects are encountered.

IJs leave their bacterial symbionts into the haemocoel of the pest and turn to 4th stage juveniles and adults. The insect host dies primarily, because of septicemia (Forst et al., 1997) (Figure 3a, b).

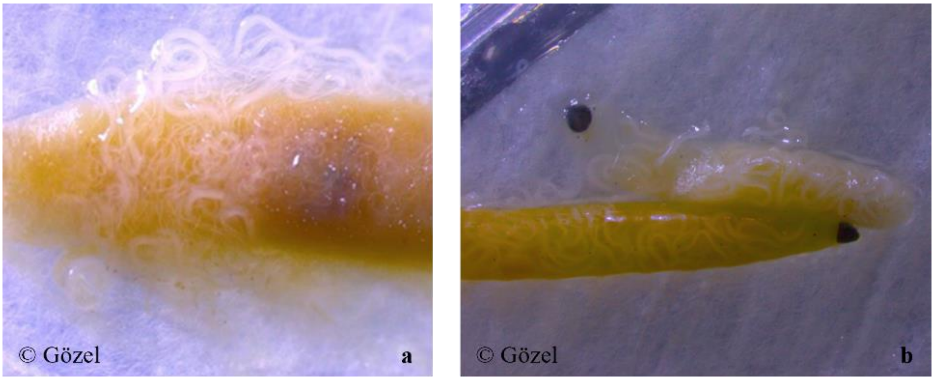

Figure 3: a. Entomopathogenic nematodes emerging from *Ceratitis capitata* larva **b.** from *Lycoriella* spp. larvae

These bacterial symbionts help to the symbiotic relationship by creating and maintaining favorable environment for nematode development. They also provide nutrients and antimicrobial materials that prevent the development of microorganisms and secondary invaders (Akhurst, 1982; Boemare et al., 1996).

IJs penetrate the pest by entering the mouth, anus, spiracles of the host or via intersegmental membranes of the cuticle (in some species) (Bedding & Molyneux, 1982; Kaya & Gaugler, 1993; Abdel-Razek, 2003). EPNs produce thousands of new juveniles that may undergo several life cycles within a single host insect (Figure 4).

Figure 4: Infected larva with full of entomopathogenic nematodes (photo provided by Randy Gaugler)

Reproduction and development within the cadaver can last 1-3 weeks when a pest is infected by EPNs in the soil and the infected pest generally dies within 24-48 hours due to bacterial toxins (Stock, 1995) (Figure 5).

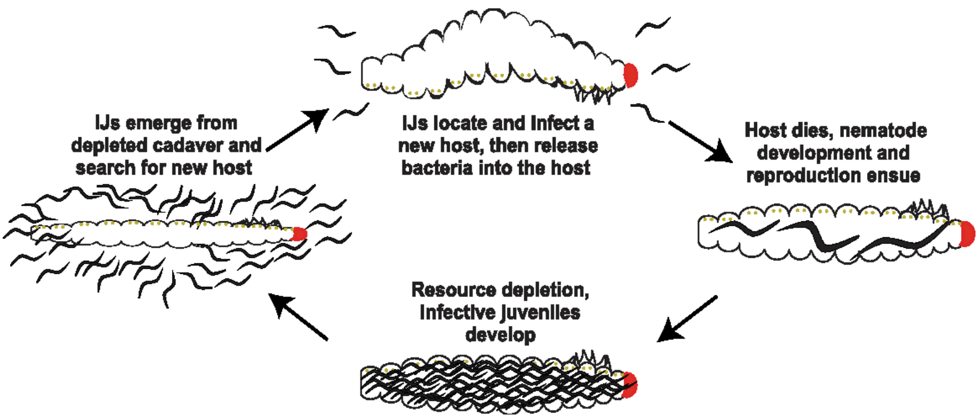

Figure 5: Life cycle of entomopathogenic nematodes (Dillman et al., 2012)

Steinernematids and Heterorhabditids have similar life cycles, the only difference occur in the 1st generation. Heterorhabditids are hermaphroditic and can reproduce in the lack of congeners, whereas Steinernematids are amphimictic, meaning they require both males and females.

EPNs live in the soil naturally and find their host in reply to carbon dioxide, vibration, and other chemical signals. They respond to chemical stimuli or feel the physical structure of insects (Kaya & Gaugler, 1993). These nematodes multiply until the food source is limited at which time they develop into IJs.

The nematodes have four juvenile stages until they become adults and many IJs are dispersed into environment to find new hosts and survive (Kaya & Gaugler, 1993). The host cadaver turns to red colour if the pests are infected by *Heterorhabditis* spp. and brown or tan colours if the pests are infected by *Steinernema* spp. (Figure 6a, b).

Figure 6: a. Heterorhabditids infected cadavers **b.** Steinernematids infected cadavers

Entomopathogenic nematodes foraging strategies vary by species, but they mainly use two strategies as ambushers or cruisers (Grewal et al., 1994). Ambushers have an energy-conserving behaviour and lie in wait to attack mobile insects in the topsoil. *Steinernema carpocapsae* is an example of ambushers. Cruisers are very active and usually subterranean, moving considerable distances by the help of volatile cues and other ways to seek their host in the soil. *Heterorhabditis bacteriophora* and *Steinernema glaseri* are examples of cruisers. Other species, like *Steinernema riobrave* and *Steinernema feltiae* prefer an intermediate foraging strategy for finding the hosts which is a mixture of ambusher and cruiser.

Many factors affect the choice of EPN to be used for controlling a specific pest insect. These are the nematodes host range, host-seeking strategy, tolerance of environmental factors and their effects on EPNs efficacy and survival (Campbell et al., 2003). Temperature, moisture, infectivity on the targeted insect and host-seeking strategy are the most important ones (Kung et al., 1991; Grewal et al., 2005). EPNs activity, pathogenicity and survival can be significantly affected by soil structure, due to its effects on moisture retention, oxygen source and texture (Gaugler, 1990; Koppenhöfer & Fuzy, 2006).

EPNs which have mobile host-seeking strategy (cruisers) are considered for use in subterranean and certain above-ground habitats (foliar, epigeal and cryptic habitats) at optimum temperatures, adequate moisture and a susceptible host while EPNs with sit and wait foraging strategy (ambushers) are the most successful in cryptic and soil surface habitats (Lacey & Georgis, 2012).

1.2. Advantages of Entomopathogenic Nematodes

- These beneficial organisms have numerous advantages. It has been proven that EPNs and their mutualistic bacteria are safe for warm-blooded vertebrates, including humans because they have no adverse effect on mammals (Poinar et al., 1982; Boemare et al., 1996).
- Most agents used in biological control require days or weeks for infecting and killing the pest, but for nematodes generally 24-48 hours are enough for killing their insect host and digest its tissues.
- EPNs are simple and relatively cheap to culture, live in the infective stage for several weeks to months and can control many important pests (Kaya & Gaugler, 1993; Griffin et al., 1990).
- It is not necessary to use masks or other necessary equipment for safety during EPNs application like pesticide application (Gaugler, 2002).
- EPNs are usually excluded from the registration requirements of pesticides in most regions of the world because of their high level of safety to the environment, non-target organisms, and humans.

1.3. Disadvantages of Entomopathogenic Nematodes

- EPNs have some disadvantages including their susceptibility to extreme temperatures, low moisture conditions and UV radiation.
- While cold-blooded species were reported as susceptible to EPNs at very high doses under experimental conditions, no adverse results were observed in the field (Poinar & Thomas 1988; Georgis et al., 1991; Bathon, 1996).

1.4. Application, Formulation and Commercial Use of Entomopathogenic Nematodes

The main point in EPNs application should be the minimum loss of IJs from the application tool to targeted pest insect. These nematodes can be applied by the equipment of other control agents but to get a cost-effective management, techniques need to be optimized for the application for a cost-effective management (Wright et al., 2005). Tools that include all agricultural and horticultural ground equipment like mist blowers, pressurized sprayers, electrostatic or aerial sprayers can be used for EPNs application (Shapiro-Ilan et al., 2006). The key points while handling an application equipment are; nozzle type, volume, pressure, agitation, recycling time, spray dispersion model and environmental conditions of the system (Lara et al., 2008).

Adjuvants can be added in the suspension for spraying because EPNs are sensitive to desiccation. Adjuvants slow down the evaporation by changing the surface tension of the applied solution. In order to avoid the groundwater and environmental contamination, it is better using natural adjuvants like vegetable oil or sugarcane molasses (Cunha et al., 2010).

Pathogenicity of these beneficial nematodes as insecticidal agents has been examined on many economically important insects by many scientist throughout the world. The number of EPN species/strains determined found successful in the studies and included in the commercialization is ever increasing. Recently commercialized EPN species is given in Table 1.

Table 1: Commercialized entomopathogenic nematode species and the continents where they have been commercialized.

***Steinernema* spp.**	**Continent***	***Heterorhabditis* spp.**	**Continent**
S. carpocapsae	AS, AU, EU, NA, SA, AF	*H. bacteriophora*	EU, NA
S. feltiae	EU, NA	*H. indica*	NA
S. kraussei	EU, NA	*H. marelata*	NA
S. kushidai	AS	*H. megidis*	EU, NA
S. longicaudum	AS	*H. zealandica*	AU, NA
S. riobrave	NA		
S. scapterisci	NA		
S. scarabaei	NA		

***AS:** Asia, **AU:** Australia, **EU:** Europe, **NA:** North America, **SA:** South America, **AF:** Africa

Remarkable success has been recorded on soil-borne insects or insects found in cryptic habitats, like galleries (e.g. *Tuta absoluta*) (Figure 7) where IJs have perfect medium to survive and protect themselves from negative environmental factors.

Figure 7: *Tuta absoluta* larvae in galleries they make on tomato leaves

In different habitats, different success rate of EPNs against various pest insects were reported by many studies. EPNs commercially used or promising against their major pests are listed in Table 2.

Table 2: Entomopathogenic nematodes commercially used or promising on crops against major target pests[1,2].

Key Crops	Pest Order	Common Name	Scientific Name	EPN Species*
Berries		Black vine weevil	*Otiorhynchus sulcatus*	*Hb, Hd, Hmeg, Hm, Sc, Sg, Sf*
Berries	Coleoptera	Strawberry root weevil	*Otiorhynchus ovatus*	*Hm, Sc*
Cranberry		Cranberry rootworm	*Rhadopterus picipes*	*Hb*
Blueberries		Scarab grubs	Many species	*Ssc*
Cranberry	Lepidoptera	Cranberry girdler	*Chrysoteuchia topiaria*	*Sc*
Banana		Banana weevil	*Cosmopolites sordidus*	*Hb, Sc, Sf*
Citrus		Citrus root weevils	*Pachnaeus* spp.	*Hb, Sr*
Citrus		Diaprepes root weevil	*Diaprepes abbreviatus*	*Hb, Hi, Sr*
Pecan	Coleoptera	Pecan weevil	*Curculio caryae*	*Sc*
Fruit trees		*Plum curculio*	*Conotrachelus nenuphar*	*Sr*
Palms		Red palm weevil	*Rhynchophorus ferrugineus*	*Sc*
Stone fruit		Flat-headed rootborer	*Capnodis tenebrionis*	*Sf*
Citrus		False codling moth	*Thaumatotibia leucotreta*	*Hb*
Nut/fruit trees	Lepidoptera	Navel orangeworm	*Amylois transitella*	*Sc*
Fruit trees		Clearwing borer moths	*Synanthedon* spp., Others	*Hb, Sc, Sf*

Pome fruit		Codling moth	*Cydia pomonella*	*Hz, Sc, Sf*
Various fruit	Diptera	Fruit flies	Various	*Hi, Sc*
Vegetables	Coleoptera	Corn rootworms	*Diabrotica* spp.	*Hb, Sc*
Vegetables	Diptera	Leafminers	*Liriomyza* spp.	*Sc, Sf*
Tomato		Tomato leafminer	*Tuta absoluta*	*Hb, Sc, Sf*
Vegetables		Armyworms	Various	*Sc, Sf, Sr*
Vegetables		Black cutworm	*Agrotis ipsilon*	*Sc*
Vegetables	Lepidoptera	Corn earworm	*Helicoverpa zea*	*Sc, Sf, Sr*
Vegetables		Turnip cutworm	*Agrotis segetum*	*Sc, Sf*
Artichoke		Artichoke plume moth	*Platyptilia carduidactyla*	*Sc*
Sugarbeet		Sugarbeet weevil	*Temnorhinus mendicus*	*Hb, Sc*
Sweet potato	Coleoptera	Sweet potato weevil	*Cylas formicarius*	*Hb, Sc, Sf*
Mushrooms	Diptera	Fungus gnats	Various	*Sf, Hb, Hi*
Greenhouse	Thysanoptera	Western flower thrips	*Frankliniella occidentalis*	*Sc, Sf*
Mushrooms	Diptera	Fungus gnats	Various	*Sf, Hb, Hi*

[1]Revised after Koppenhöfer et al. (2020).

[2]At least one article reported ≥ 70% control of pest in the field; this table is not prepared as a detailed list.

*****Hb: Heterorhabditis bacteriophora*; *Hd: H. downesi*; *Hmeg: H. megidis*
Hm: H. marelata; *Hz: H. zealandica*
Sc: Steinernema carpocapsae; *Sg: S. glaseri*; *Sf: S. feltiae*
Ssc: S. scarabaei; *Sr: S. riobrave*

Different factors including IJ concentrations, additives, carriers, moisture content, temperature, oxygen, and UV-ray protectors play important roles in formulations of EPNs. Aqueous suspension, alginate capsules, dispersible granules, sponge, and clays are mostly used formulations of commercialized EPNs but the formulation type can be changed based on the EPN strain and culture methods (Grewal, 2002; Hussein & Abdel-Aty, 2012; Guo et al., 2017; Heriberto et al., 2017).

2. CONCLUSIONS

Biological control is an activity to reduce the damages of the pests on the products by using the natural enemies of these pests. For a successful biological control in agricultural ecosystems it is crucial to comprehend the adaptability and sustainability of applied biological control agents (Flint & Dreistadt, 1998). The use of EPNs which are considerable natural enemies of soil-borne insects is a non-chemical way for controlling pests but each nematode species may have differences in infectivity, survival, and searching approach so they can be more or less proper against a specific pest in management programmes (del Pino & Palomo, 1996). These beneficial organisms are used as alternative biological control agents in various researches to suppress many significant pests all over the world (Jansson et al., 1993; Enrique Cabanillas & Raulston, 1995; Shields et al., 1999; Shapiro & McCoy, 2000; Morton & García-del-Pino, 2009; Shapiro-Ilan et al., 2010; Gozel & Gunes, 2013; Gozel & Kasap, 2015; Gozel & Genc, 2021; Abbas, 2022).

Pairing the proper nematode host-finding strategy with the insect is one of the key points of this control. The most usual mistake observed in the EPN application is poor host suitability (Gaugler, 1999). Prior to EPN application in the field, abiotic factors such as soil type, soil temperature and moisture are necessary for a successful pest management (Kaya & Gaugler, 1993). Also application basics like field dosage, volume, irrigation and correct spraying method have great importance. Additionally plants morphology and phenology should be considered in predicting whether EPNs are viable control agents (Georgis et al., 2006).

Native EPN species may consider as a precious resource, not only from a biodiversity perspective but also from an environmental perspective due to their ability for adaptation to regional environmental conditions. It is clear that the utilization of native EPN species/strains can be an eco-friendly alternative to chemical pesticides and match perfect with IPM but further studies are required to define new native EPN species (Gozel & Gozel, 2016). Consequently, all possible opportunities to optimize EPN study areas and to better use as biopesticides should be exploited but end-user education and marketing support is necessary to widen the utilization of these beneficial organisms in pest control programmes.

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CHAPTER 9

THE NON-DESTRUCTIVE TECHNIQUES USED TO DETERMINE THE QUALITY CHARACTERISTICS OF AGRICULTURAL PRODUCTS

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INTRODUCTION

High quality and safety are among the most sensitive points of end users in agricultural products and foods. Especially under the pandemic conditions, customers pay more attention to these important issues regarding food safety. In addition, there are extremely strict analyses and laws regarding food safety in the export of agricultural products. In this context, product quality and quality criteria constitute a critical point in the eyes of the consumer. Agricultural production aims to obtain maximum yield and the best quality products with minimum input at every stage of cultivation. Today, it is necessary to minimize today's problems by using smart agriculture applications, which is one of the technological developments in the field of agriculture when the need for food is important. Smart farming techniques can be used in almost every period of plant production, from tillage to harvest. It is desired that the maturity of agricultural products and internal and external qualities be well preserved in the stages from the harvesting process until they reach our table. During the transmission process from the field to the table, mechanical damages generally occur in the products due to the external environment. These damages reduce the storage time of the products and reveal microbial effects (He et al. 2022). In determining the quality characteristics of fruit and vegetable products, features such as shape, color, brightness, texture, and taste come to the fore. Quality analyses of these properties are still carried out by giving damage to the food products or using chemicals in the food industry (Narayan and Matsuoka 2000). Analyses to determine the quality characteristics of agricultural products are usually expensive, time-consuming, and damaging to the products (El-Mesery et al. 2019). Internal quality characteristics such as maturity, sugar, acidity, and internal damage may be more difficult to predict. At the same time, because different people perform different analyses, the accuracy can be varying. With the development of technology, damage-free detection systems can perform such analyzes without damaging the products. In today's world, the use of such systems can partially prevent food waste, which contribute to meet the increasing population's food needs.

This chapter summarizes recent non-destructive technologies widely used to determine agricultural products internal and external quality.

1. Near infrared Spectroscopy

Near-infrared technology has been used in chemistry, polymer, pharmaceutical, and even more industries in recent years. In factorization, NIR spectroscopy could rapidly and non-destructively check the internal and external properties of the raw materials and test the final products before the delivery to the customers. NIR spectroscopy has emerged over the years in related industries with these features. For non-destructive qualitative and quantitative analysis of raw materials, in-process materials, and finished products throughout the manufacturing process. For this purpose, NIR spectroscopy is also widely used in the food industry (Niemoller, A. & Behmer, D., 2008).

The main advantages of NIR spectroscopy:

- While sample products have to be prepared for quality analyses in the chemometric analyses but there is not necessery in the near infrared spectroscopy.
- The samples used during the analysis of the products are deformed and cannot be used. Since spectroscopic techniques are optically based technologies, there is no residue in the products and microbial effects that may occur afterward can be prevented.
- Quality analyzes can take a long time when done with chemometric methods. However, it is possible to get results in a short time with spectroscopic methods.
- Spectroscopic analyzes are measurement methods with high accuracy besides being fast. Since no damage was done to the samples during the analyses, the analyzes were repeated.
- In terms of cost, the installation costs can be high, but the costs can be low for long-term use. In addition, since no chemicals are used, the analysis costs are pretty low.
- Spectroscopic methods can prevent human-induced errors.
- Today, with the advancement of technology, portable spectroscopic devices are produced and measurements can be made in the field environment.

This section gives an overview of NIR technology, principles and its equipments and how to describe raw material specifications non-destructively.

1.1.Near Infrared Spectroscopy Technology

Near infrared spectroscopy, occurs the absorbance of radiation at molecular vibrational frequencies (Soriano-Disla et al. 2014). It can also be expressed as an energy exchange between matter and light. The vibrations in the functional groups of the samples are located in the NIR (1000-2500 nm) spectral region. C-H (aliphatic), C-O (carboxyl), C-H (aromatic), O-H (hydroxyl) and N-H (amine and amine) organic substances in the organic compounds of the products are located in the NIR region due to their strong absorption and combination modes. This creates fingerprints in the spectral regions of organic compounds (Zou and Zhao, 2015). Therefore, each product differs from the other due to its unique characteristics.

At certain ambient temperatures, many molecules are at fundamental vibrational levels. Atoms and groups of atoms participating in chemical bonds can be replaced by each other at the frequency they are defined, depending on the strength of the bonds or the mass of the bonded atom. Compared to the bonds of heavy atoms, bonds with light atoms vibrate at higher frequencies. When looking at C-H, C-D, C-O, C-Cl and C-Br bonds, it is seen that they vibrate at frequencies of 3000, 2280, 1100, 800 and 550 cm-1, respectively (Dufour, 2009).

This system non-destructive systems; which were used for to determine the quality features of agriculture foods, are developed using UV-visible devices in near infrared spectroscopy. Compared to the devices used in other infrared regions, such applications can be low-cost. The most commonly used detectors in NIR type devices are InGaAs types. The advantages of these detectors are that they can get results quickly and have high estimation features. In InGaAs type detectors, tungsten or halogen lamps with a high radiation source are generally used. Because these lamps have a high signal ratio, they are preferred in NIR devices (Pasquini, 2003). Monochromators are used to measure NIR spectra and the entire visible region in reflection and transmission modes. Monochromators come in three types: silicon (400-110 nm), indium gallium arsenide (800-1700 nm) and lead sulfide (1100-2500 nm) detectors. This type of monochromators can be used separately according to the structural properties of the products (powder, solid or granular) or combined with fiberoptic probes for surface reflection measurements or transmission measurements (Osborne, 2006).

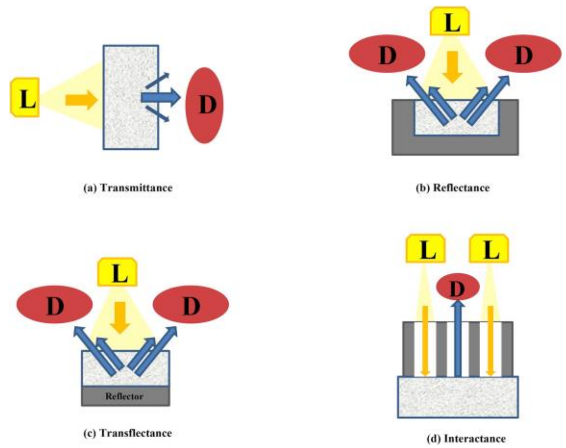

Figure 1: Different modes of acquisition of spectra (Cortés et al., 2019)

Near infrared spectroscopy includes various optical measurement modes such as transmittance, reflectance, transreflectance and interactance (Figure 1). The status of light sources and detectors varies according to the measurement modes (Cortés et al., 2019). In reflection modes, information can be obtained with the outer surfaces of agricultural products (shell, color, etc.). In contrast, information about the internal properties of the products (sugar, soluble solid content, etc.) can be obtained in the transmission mode.

Near infrared spectroscopy can monitor undamaged agricultural products and is one of the most powerful technological applications in recent years in predicting various quality and safety characteristics. When combined with chemometric applications, it appears as a fast, undamaging, and inexpensive method for determining the quality characteristics of agricultural products and can be used as an alternative method to damaged analyses.

2. Hyperspectral imaging

Hyperspectral Imaging is among the systems that can measure quality properties without damaging products such as near infrared spectroscopy. Hyperspectral image detection, which is included in remote sensing (contactless sensing) technology, is the measurement of the energy reflected from the object (organic/inorganic) surfaces in narrow and adjacent multiple wavelength bands and the recording and processing of data from the electromagnetic spectrum. The difference between hyperspectral imaging from other spectral systems is that it detects and evaluates the features, such as spatial information, spectral information, sensitivity to minor components and building chemical images (Feng & Sun, 2012). Hyperspectral imaging relies on optical reading. All biological materials have different molecular bonds and forces that hold tissues together. Carbohydrates, water, and fats are rich in O-H and C-H bonds, and organic compounds are rich in C-H and N-H bonds (Huang et al., 2014).

Spatial images obtained in hyperspectral imaging consist of different wavelengths of the objects. The resulting hyperspectral image is obtained by superimposing spatial images collected by hyperspectral sensors. Thus, it enables the creation of a three-dimensional data cube, called a hypercube, which is further analyzed and displayed. A hyperspectral cube is a composite vector containing spectral information of various wavelengths. With the obtained pixel vectors, definitions or predictions can be made about the properties of the products (Wu and Sun, 2013a).

When we look at the entire optical system in hyperspectral imaging, lenses for spectral and spatial images, imaging spectrographs and CCD or CMOS cameras for 2D measurements are needed for applications. The most important part of the system is the spectrograph. A spectrograph is a system that transmits multiple images of an illuminated entrance slit to a photosensitive surface (detector). The position of the images is a function of wavelength. It is generally characterized by the absence of moving parts (El-Mesery et al., 2019).

Hyperspectral imaging is used to absorb the light coming into the sample to estimate the sugar content in the fruits and vegetables. In contrast, the scattering of the incoming light is affected by the cell structure, particle sizes and density in the product (Siche et al., 2016). Hyperspectral imaging can be used as the most powerful technique to perform the quality and analysis of biological materials such as food products, to determine their morphology, to have information about their chemical components without damaging the products.

3. Computer Vision

Packaging or classification of agricultural products brought to the factory after harvesting is done according to the quality or dimensional characteristics of the products. Since humans usually perform such operations, there may be a waste of time or cases where accurate results cannot be achieved in the analyses made. In addition, there may be errors arising from the concentration disorder of the employees in the classification processes. With the improving of technology in the agriculture and food industry, automatic control systems keeps developing. In particular, the evaluation of the final quality of the products and the determination of their texture, color and dimensional properties can be done with optical systems more efficiently, quickly, cheaper and without damage. For this reason, computer vision techniques widely used for classification of the food products.

After the agricultural products are harvested, they are subjected to many processes such as classification and packaging in the factories. In the fabrication processes, the products collected from the field are classified according to their quality characteristics before they reach the end user. Especially in the packaging stage, by using the computer vision technique, the properties of the products can be determined without a damage and the quality control of the packaged products can be ensured. Computer vision technology can be explained as the identification and evaluation of physical objects from the images of agricultural products. The system comprises five main elements: a frame grabber, a camera, illumination source an image and hardware-software (Figure 2). Illumination significantly impacts the quality of the captured images but also affects the overall success of the system. Well-designed illumination system increases the success of image analysis. Efficient illumination reduces the reflection of the products and helps shorten the processing time by eliminating shadows and some noises. The location, lamp type and color quality are important in designing the lighting. While lamps such as fluorescent, lasers, X-ray, and tubes are generally preferred for lighting, the choice of the lamp can affect the image processing performance (Brosnan and Sun, 2004).

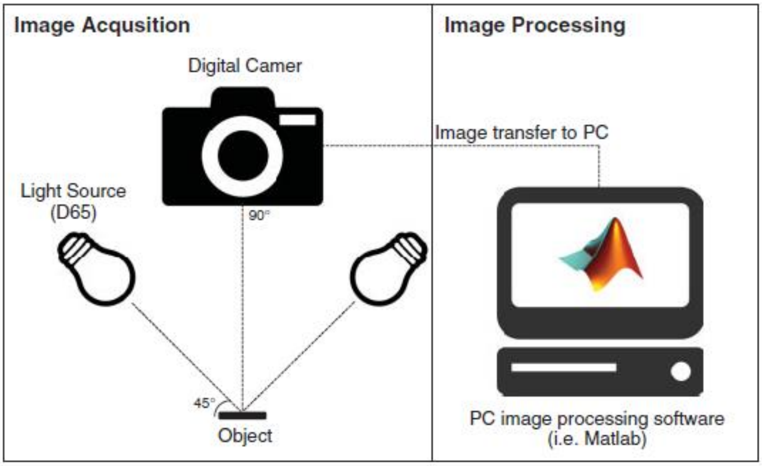

Figure 2 : The parts of computers vision systems (Mogol and Gökmen, 2014)

Digital images can show spatial and density (color) properties of the products. While images are expressed numerically with a 2-dimensional matrix consisting of rows and columns, a small area is called a pixel. The color space, on the other hand, is a 3D model and allows the color to be examined in three different directions (RGB, Lab…). In a digital image, each pixel digitally generates the colors, red (R), green (G) and blue (B), depending on the matrices. through the color matrices obtained in this context, the differences in agricultural products can be digitized and information about the products can be obtained (Mogol and Gökmen, 2014). Generally, solid state charged coupled device (CCD) or complementary metal-oxide semiconductor (CMOS) cameras are used in the image processing systems. CCD cameras consist of many photodiodes (pixels) made of photosensitive materials that can read the transmitted light energy as an electronic charge. In CMOS cameras, each pixel has a photodetector and a reading amplifier. Cables in CMOS cameras can quickly transfer the signals they obtain. Therefore, it is most used in the areas that require more high-speed imaging. It is one of the most critical parts used in the image processing system because it is cheap, consumes less energy, contains a single power source, and takes up little physical space in the system (Wu and Sun, 2013b). Computerized imaging has many advantages such as providing measurements which is fast, cheap, predictable, objective, effective and undamaged, needing no sample preparation processes, enabling to analyze every pixel of the outer surface of food products, and measuring the products in every which way. These systems, which can be used especially in the manufacturing stages of the food industry, add importance to the packaging and quality processes of food products.

4. Electronic Nose

Past analyzes have relied on the human experience. However, human noses can quickly get sick due to external environmental conditions or illnesses. The electronic nose is a method in which chemical sensors detect complex organic compounds and flavors in the food industry. In addition to the undamaged detection of volatile components in foods, electronic nose technology is used in many different fields, such as medicine, safety, and the detection of biomedical diseases. High accuracy estimation and good quality results were the advantages of the electronic nose system when compared with the sensory analysis (Shi et al. 2018).

Generally, the electronic nose system consists of three main systems: the sensors that detect volatile substances, the format that converts the odor detected by the sensors into signals, and the software in which the necessary analyses are made. Statistical methods are used to distinguish the differences between the samples in line with the analysis results obtained (Mahmoudi, 2009).

There are four types of sensors:

• **Catalytic or tin oxide sensor:** Taguchi gas sensors are generally used for the main detection element in the inline odor detectors. It is produced from a ceramic pellet on which a tin (II) oxide film is formed and heated by electricity. Tin (II) oxide film can produce the negative charged oxygen related to temperature where oxygen absorbed in the surface of the products Thus, the electronic nose can generate signals with its negative oxygen. Taguchi sensors generally require high temperature and power consumption during operations.

• **Conducting polymer sensors:** Many sensor materials are either conductive or semiconductor. It changes in conductivity with the absorption of various gases and vapors. Polypyrole and polyaniline are conductive polymers used in liquid and gas phase sensors. Their advantages are low power consumption, rapid absorption in their sensors, customizable polymer structure, and sensitivity to moisture.

- **Acoustic wave sensors:** AT-cut quartz crystals can be used as piezoelectric sensors in the system due to their high temperature parameters. The type of acoustic wave produced in piezoelectric materials can be determined by the crystal cut, the thickness of the material used, and the geometry and configuration of the metal electrodes used to generate the electric field.
- **MOSFET technology:** Although it is a high-impedance transistor, the most sensitive measurements can usually be made by using this technology (Deisingh et al., 2004).

With the interaction of aroma changes, volatile compounds and conductive polymer surfaces in the products, changes in electrical resistance occur and sensors can detect these changes. Thus, the detected signals can digitize the received change and enable statistical analysis. The obtained signal values help in a model development by using data processing techniques such as Cluster Analysis (CA), Principal Components Analysis (PCA), Discriminant Function Analysis (DFA), Neural Network, and Fuzzy Logic (Mahmoudi, 2009).

Nowadays, the electronic nose is mostly used in the food industry. It is also essential to assessing the quality of products, especially in the food production stages. Being non-destructive and fast are among the most significant advantages of the system. The shelf life of food products is also important in terms of quality. The electronic nose applications can evaluate fruit and vegetable qualities based on aroma properties. In addition, there are areas of use in the determination of the harvest time, detection of diseases, identification of cultivars, development of perfume and cologne products in the field of cosmetics as well as in the health and medicine, and various other fields (Wilson and Baietto, 2009).

5. Acoustic Methods

Acoustic analysis, like other non-destructive techniques, can be used to determine food quality. One advantage of the system is that it can analyze it quickly, with a high degree of accuracy, economically and without causing a damage. Applications in the acoustic technology are similar in the form of scattering and reflection of sound waves, just like light waves. The acoustic pulses are sent to the product with the help of a transducer and the pulses from the product are transmitted, scattered or reflected. Acoustic emission is vital in terms of food texture. Acoustic parameters can provide information about the freshness of the products, especially in snack-style (crispy, crispy properties) products (Aboonajmi et al., 2015).

By analyzing the sound responses of mechanical impacts applied to fruits at specific frequencies, information about the internal quality; such as water content, flesh texture, firmness, crispiness, of the products can be collected (Aboonajmi et al., 2015). Algorithms are developed with the help of the obtained frequencies, and the differences between the products can be detected more clearly with the help of artificial neural networks. In this direction, quality assessment can be made quickly and without damaging the products (Lashgari et al., 2017).

It clearly shows that the usefulness and economics of the acoustic method makes it to be one of the most significant benefits in the agri-food industry. In parallel with the widespread use of computers and electronics, the application and correctness of acoustic techniques and the speed of data processing will increase, and online decisions on the quality assessment system will be made. The acoustic method, among other modern methods, provides information on the nature of destruction processes and enables the onset of these events before maximum material stress occurs. The advantages of non-destructive acoustic techniques make it very likely to be widely used in the agri-food industry soon.

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CHAPTER 10

EVALUATION OF THE USAGE OPPORTUNITIES OF THE URBAN FORESTS AS A RECREATION AREA; EXAMPLE OF ALIAGA DALGAKIRAN B TYPE RECREATION AREA

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INTRODUCTION

Despite the increasing importance of the rural landscape, the importance of green areas in and around the city and the psychological needs of the citizens for these areas are increasing due to the increasing air pollution in urban areas, climate change and urbanization pressure. While only 9% of the world's population lived in cities in 1900, this value increased to 40% in the 1980s, 50% in 2000, and 70% today. This increase in urbanization has started to cause people to be disconnected from nature and to increase the stress level, various diseases and psychological problems due to air pollution and temperature increase. Apart from the recreational needs of the citizens; in order to meet the health, sports, aesthetic, cultural and social needs and to enrich the flora and fauna, the need to create forest areas in the cities has emerged. For this reason, different disciplines carry out many studies to increase green infrastructure in cities. Urban forests are areas that improve the quality of life of the people living in the city, increase the economic value of residential and commercial areas, regulate air quality, reduce carbon dioxide accumulation and the effect of hot air in urban areas, and contribute to the mental and social comfort of the society.

The criteria that green areas created in the city should have in order to be considered urban forest were examined in the literature in this study, and it was determined whether the Dalgakıran Type B Recreation Area, located in Section 52 of the Aliaa Forestry Operations Directorate's Management Plan, which will be constructed in accordance with these criteria, meets these criteria.

Nature, which is of great importance for both physical and mental health of human beings, is being killed more and more by human hands every day. However, as the negative impact of the destruction on people is realized over time, the importance of the existence of green spaces in our cities, which are in the form of concrete piles, is understood. Issues such as global warming, climate change, air pollution, etc. are also discussed in relation to each other. In this direction, many different disciplines are working on the development of green and blue infrastructure in cities, and the effective use of irresponsibly consumed natural resources and sustainability come to the fore.

The concept of urban forest has entered the literature in this context and is one of the green infrastructure elements. The forests, which initially functioned as wood production, have led to the emergence of many new forestry types with the diversification of the needs for the benefits and functions offered by the forests in the society over time. These are classical or traditional forestry, social forestry and urban forestry (Asan, 1999). The concept of "urban forestry" was first used in a study conducted at the University of Toronto in 1965 on the success and failure of local government plantations (Turna, 2017). In 1972, the Forestry Association of America established an 'Urban Forestry Working Group'. According to the definition made by the 1972 American Forestry Association; Urban forestry is a specialized branch of forestry that aims to manage and grow trees for their current and potential contribution to the psychological, social and economic comfort of urban societies (Kielbaso, 2008). The beginning of the concept of urban forest in Europe was in the 1980s, with studies in England and the Netherlands (Raundrup et al., 2005). In England, the National Urban Forestry Unit (NUFU) was established as an independent organization in 1995 (NUFU, 1999). Ireland became the second country after the UK to adopt the concept and the first urban forestry conference was held in Dublin in 1991. Afterwards, the subject has been discussed in many studies by different disciplines in Europe (Saglam & Elvan, 2017).

Miller (1997) urban forestry; "The technology, science and art of managing forest resources and trees in or around urban community ecosystems, providing aesthetic, economic, psychological and sociological benefits to society." defined as. Konijnendijk (2003) states that urban forest includes not only forests around and within urban areas, but also other trees and related vegetation (large trees in parks and roadsides, agricultural fields and other private land). Coşkun & Velioğlu (2004); they defined the urban forest as "green areas that serve the urban identity, texture and the quality of life of the citizens in or around the urban ecosystem". According to Geray (2003), Urban Forestry is made up of forest ecosystems, woodlands, trees, shrubs and trees in order to secure sustainable urban life with its history, architecture, texture, organization and culture and to contribute to the physiological, psychological, economic, social and morale level of the urban society and to benefit from the bushes and to protect, develop and manage these resources".

In our country, where the majority of the population lives in cities, the loss of green space as a result of unplanned urbanization has aggravated societal problems in terms of physical and mental health. (Uslu & Ayaşlıgil, 2007). The increase in the need for green areas in cities, as well as the diversification of the expected benefits, were handled by the relevant ministry, and in this context, the General Directorate of Forestry began to implement the "Urban Forest Project" in 2003 (Atmiş, 2016). However, the legal definition of the urban forest was made for the first time in article 4/ğ of the abolished Recreation Places Regulation. The Regulation on Recreation Areas, which is in force today, has redefined the types of recreation areas and redefined the urban forest. According to article 3f of the regulation, D type urban (city) forest: “To put the health, sports, aesthetic, cultural and social functions of forests into the service of the public, at the same time to contribute to the beauty of the country, to meet the various sports and recreational needs of the society, to allow touristic activities and to provide technical With the aim of promoting forestry activities and flora and fauna and instilling the love and awareness of forest among children and young people, scouting, trekking, cycling, horse riding and similar activities, country restaurant, country coffee, culture houses, local product exhibition and sales place, amphitheater, means the places allocated in provinces and districts, containing various mini-sports fields and other recreational structures and facilities.

The aim of this study is to examine the criteria of being an urban forest in our country and in the world, and to examine whether the Dalgakıran Project, which is being built in Aliağa and is a forest area according to the land registry records, can be qualified as an urban forest in the light of these criteria. With work; Based on the Aliağa example, the definition of urban forest in our country has been re-evaluated.

MATERIAL AND METHODS

Working Area

Dalgakıran Type B Recreation Area is located in the Kültür District of Aliağa, on an area of 4,5076 ha, at an altitude of 36 m in the çfa-1 stand type in the division 52 in the Aliağa Forestry Operations Directorate's management plan. The first registration date of the recreation area is 03.06.2015/118. It is 80 km away from İzmir city center and 1150 m away from Aliağa. It is 1 km away from Aliağa Promenade, which is the closest recreation area. Its property is forest land. As seen in Figure 1, the working area is a part of the forest area that remained in the city. Figure 2 shows the relationship of the area with the city center.

Figure 1: Location of the study area

Figure 2: Photo from study area

Urban Forest Criteria

Urban forest criteria in our country have been determined with the Recreation Places Regulation dated 05.03.2013 and the Recreation Places Implementation Communique dated 31.12.2014, which was prepared as a basis for the implementation of this regulation. According to this;

- Except in cases of necessity, A and B type recreation areas are minimum 2.0 ha, maximum 50.0 ha., C type recreation areas minimum 1.0 ha. maximum 20.0 ha, urban forest minimum 5.0 ha, maximum 300.0 ha.

- Although it is essential that the proposed area for the recreation area be in one piece, a reasoned report will be prepared if a proposal is made for the establishment of a 2 or 3 piece recreation area due to difficulties in protection and similar reasons.
- 1/500, 1/1000 or 1/2000 scaled site plan, development and management plan and plan reports in urban forests will be prepared or made by the relevant forest regional directorate.
- In urban forests; amphitheater, vehicle path, guardhouse, bicycle track, buffet, fountain, environmental protection, children's playground, warehouse, natural navigation bridge, natural and artificial water ponds, nature walking area, flora and fauna display area, septic tank, access control building, observation tower, prayer room, administration and visitor building, scouting activity areas, gazebo, country cafe, country restaurant, culture house, adventure park, manege and animal shelter, scenic viewing terrace, mini sports fields, firepit, forestry applications promotion area, parking lot, path, paintball field, pergola, sports facilities support building, water tank, ornamental pools, rain shelter, local products sales place, teleski and cable car line, toilet, swimming pool, flora and fauna promotion area, etc. can be done.

Apart from this, there is also a recreation potential evaluation table in the communique. In this table; detailed criteria and scores of these criteria have been determined under the headings of "Area and Landscape Value", "Climate Value", "Accessibility and Visitor Potential", "Recreational Facilities and Environment", "Negative Factors". Although not specific to urban forests, areas with high scores are selected by using this table in the selection of recreation areas in general.

In the study of Gül & Gezer (2004); have determined some basic criteria for the site selection of urban forests.

Recreational Goals:

- It is the most important criterion to be considered in the site selection of the urban forest. The urban forest should be easily accessible by private and/or public transport.
- The area should provide opportunities for various recreational activities with its potential, cultural and aesthetic values.
- In terms of vegetation, areas rich in coniferous species and broad-leaved species (mixed-leaved tree species) should be preferred as priority.
- Areas with water potential should be selected for urban forests. The presence of drinking water sources increases the attraction power of these areas. The presence of large water resources such as sea, lake, pond and river in the area is important in terms of utilization potential as well as benefiting from its visual impact.
- Existence of interesting areas and view points are other priority reasons for preference.

The Goal of Strengthening the Physical Structure of the City:

- The potential of urban open and green spaces to limit, integrate and buffer spaces should be taken into account in the selection of urban forest.
- Areas with high potential to provide organic connection and integration with open and green spaces in the city, and the natural areas in and outside the city should be preferred as a priority.

Ecological Goals:

- In urban forest location selection, areas containing water resources that can contribute significantly in ecological terms should be selected.
- Areas rich in natural values such as wildlife and plant diversity are areas that will provide significant benefits in integrating urban people who are disconnected from nature through protection and controlled use.
- In terms of topographic structure, flat and nearly flat areas with little slope are suitable for many recreational activities. In terms of land structure, flat or nearly flat areas, areas with a low slope, that is, 0-10% slope, should be preferred as priority.

- In short, urban forests should fulfill all the ecological, aesthetic and functional functions and functions of urban open and green spaces.

Gül et al. (2006) developed an urban forest site selection model using multi-criteria analysis. According to this study, the necessary criteria for an area to be designated as an urban forest are shown in Table 1.

Table 1: Urban Forest Site Selection Criteria (Gül et al., 2006)

Main criteria	Indicators	Score
1. Position	In urban development	3
	Adjacent to urban border	2
	1 < km from urban border < 5	1
2. Size	50 ⩽ ha ⩽ 100	1
	100 ⩽ ha ⩽ 1000	2
	> 1000 ha	3
3. Ownership	State or other public property	3
	Foundation property (e.g., charitable communities, associations and clubs)	2
	Private property	1
4. Existence of strict protection areas without recreational usage	Yes	1
	No	3
5. Usage by other organization or sectors	Yes	1
	No	3
6. Existence of limiting structures (e.g. airport, highway, and factory)	Never	3
	⩽2 limiting structures	2
	>2 limiting structures	1
Total possible score (expressed as a range)		6–18

Each indicator received a score between 1 (least important) and 3 (most important).

The site selection criteria determined in the study conducted by Van Elegem et al. (2002) for urban forest site selection with multi-criteria analysis are shown in Table 2.

Table 2: Urban Forest Site Selection Criteria (Van Elegem et al., 2002)

Criteria used
Criteria related to the potential recreational quality
Population density
Number of inhabitants within a radius of 3 km
Number of inhabitants living in areas without gardens within a radius of 3 km
Accessibility
on foot
by bicycle
by public transport
Absence of hindering infrastructure
Absence of heavy industry
Absence of road infrastructure
Absence of soft outdoor recreation facilities in the neighbourhood
Structure-strengthening criteria
Potential of spatial bordering of the urban area by forest
Potential buffer against spatial joining of residential areas
Sub-unit of a city green axis
Sub-unit of the natural structure
Scenic accentuation of cultural-historical elements
Degree of cohesion
Linkage of big open areas (extension facilities)
Degree of fragmentation
Potential protection function
Criteria related to the potential ecological quality
Geographical diversity within the area
Texture classes
Drainage classes
Topographical variation
Presence of forest
Current presence of forest
Presence of ancient woodlands or wood relics
Former presence of forest
Potential cohesion with existing nature values

EVALUATION AND CONCLUSION

Increasing stress in cities, air and noise pollution, unplanned construction, insufficient open green spaces, increased traffic density, insufficient infrastructure, increased stress due to deterioration in the urban economy, and as a result, the increase in the desire of the citizens to be closer to nature and green, urban forests and all open spaces increased the importance of green spaces. The number of studies on sustainable cities, green and blue infrastructure is increasing day by day.

Urban forests are naturally found or artificially established in and around the city, provide aesthetic and functional contributions to the urban structure, offer recreational opportunities to the urban people and can be reached in a short distance (Uslu & Ayaşlıgil, 2007). In most countries, trees, shrub groups, parks, forests within the city, trees located on the side of the road are also considered as urban forests. However, when urban forest is mentioned in our country, recreation areas with a size of at least 5 ha in which various activities can be carried out come to mind. The framework of this definition is drawn with the Regulation on Recreational Places and the implementation communiqué issued accordingly. Transforming the remaining forest parts or empty, idle areas into urban forests by planting trees; it is of great importance both in terms of protection and increasing green space. Urban forests not only meet the psychological, physical and recreational needs of the residents, but also have a great impact on the urban climate. These areas, which can be considered as carbon sinks, are also a solution for reducing both air pollution and the heat island effect. It is possible with such practices to produce local solutions to climate change, which has become a global problem, and to the climate problems that arise accordingly.

In this study; Aliağa Dalgakıran Recreation Area Project, which has been determined as a Type B Recreation Area according to the Recreation Areas Regulation in force in our country, has been examined. This area, whose borders were defined by selecting a small part of a large forest area remaining in the city, has an area of approximately 4.5 ha (Figure 3).

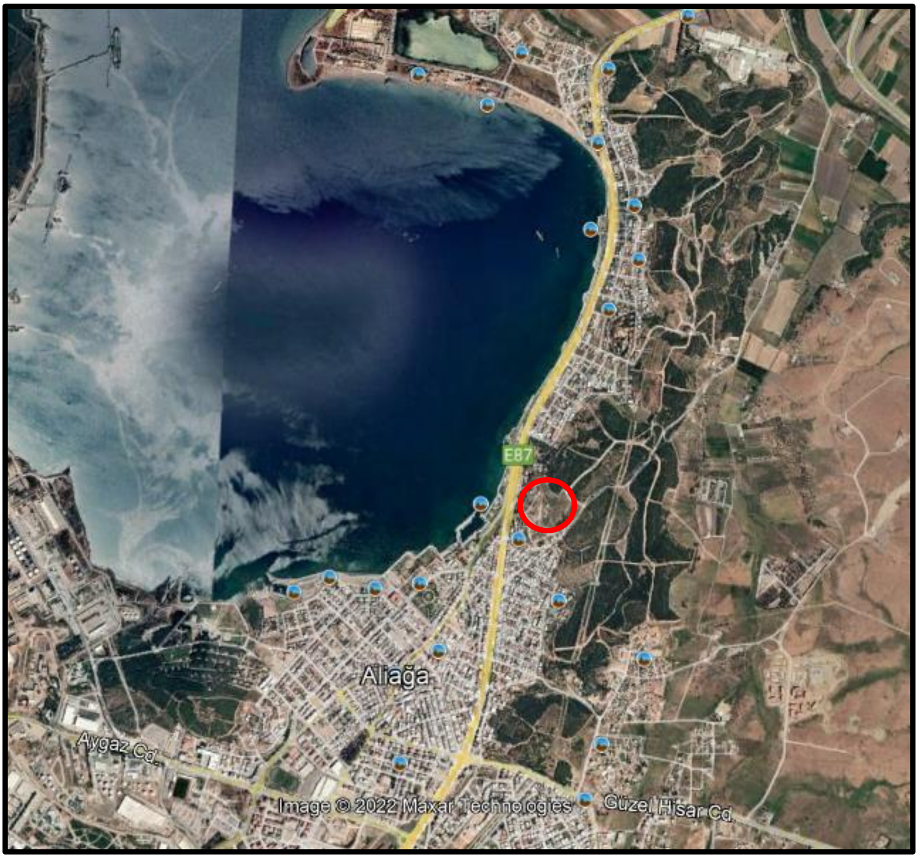

Figure 3: Study area within the forest area

Type B in the Recreation Areas Regulation; "In order to meet the various resting, entertainment and sports needs of the society, to contribute to the beauty of the country and to allow touristic activities, the country restaurant, country coffee, local products, exhibition and exhibition and regional products, with recreational resource values and 6 high visitor potential, are available only for daily use. are defined as promenade areas containing other recreational structures and facilities such as sales places, picnic units, arbors.

In the same regulation, D Type Recreation Areas, namely Urban Forests; "To put the health, sports, aesthetic, cultural and social functions of the forests at the service of the public, to contribute to the beauty of the country, to meet the various sports and recreational needs of the society, to allow touristic activities, and by introducing the flora and fauna with technical forestry activities, especially for children and With the aim of instilling a love and awareness of forest in young people, scouting, trekking, cycling, horse riding and similar activities, country restaurant, country coffee, culture houses, local product exhibition and sales place, amphitheater, various mini sports fields and other recreational structures and facilities. , provinces and districts are defined as “the places allocated.

There is no big difference in the facilities that can be built according to the regulation. However, 6.2. In the article, “Except in cases of necessity, A and B type recreation areas are minimum 2.0 ha, maximum 50.0 ha., C type recreation areas minimum 1.0 ha. maximum 20.0 ha, urban forest minimum 5.0 ha, maximum 300.0 ha. A justification report will be prepared by the commission consisting of the Branch Manager, the forest operation manager and the forest operation chief, under the chairmanship of the relevant Regional Deputy Director, for the recreation areas to be proposed different from the specified areas. Although it is essential that the proposed area for the recreation area is in one piece, a reasoned report will be prepared in case a 2 or 3 piece recreation area establishment proposal is made due to difficulties in protection and similar reasons. Since the study area is 4.5 ha, it is not defined as an urban forest.

However, when the study area is examined on the satellite photo, it is seen that the study area, which is a part of the forest texture surrounding the north of the city of Aliağa; It is seen that it has an important location with sea view due to the fact that the number of trees is the least, the closest to the city, and the peak.

Considering the legal framework and previous studies, which are examined in detail in the Materials and Methods section, it is seen that the criteria for being an urban forest are proximity to the city, areal size and transportation criteria. In this direction; The study area meets all the criteria except size, but considering that there is actually continuity of the forest texture, but this area is chosen with an artificial border, it is possible to enlarge the area.

Instead of designating this area as a B type recreation area, it should be determined as a D type recreation area, namely the Urban Forest; It will both bring a larger open green area to the city, allow more social and cultural activities, and will ensure that the degraded forest quality will be corrected with tree plantings to be made compatible with the surrounding forest texture, the diversity of flora and fauna will increase, and the climate will be affected due to the increase in the number of trees will cause positive effects.

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CHAPTER 11

POULTRY RED MITE AND INTEGRATED PEST MANAGEMENT IN POULTRY PRODUCTION

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INTRODUCTION

Poultry products are one of the basic foods in human nutrition because they are relatively cheaper and easily obtainable compared to other animal products. When the poultry production systems are examined, it is seen that intensive farming is dominant, which is generally more integrated and aimed at getting more yield from the unit area. The high number of animals housed in a unit area brings along many problems. One of them is that the possible effects of any disease or pest are likely to spread more quickly to the whole herd. The European Union HAS banned the cage breeding system with the decision taken in 2012 and made it necessary to switch to other systems, such as enriched cages or ground breeding. In comply with this decision on the basis of animal welfare, traditional housing systems will gradually change until 2027. Existing shelters will be arranged for rabbit, chicken, duck, quail and goose breeding. Shelters will be designed to allow animals to exhibit more species-specific behaviors. The shelters will be forced to have equipment such as perches, nests and litters. Although this change is positive for animal welfare, it will complicate to control of poultry red mite.

The poultry red mite (PRM, *Dermanyssus gallinae,* De Geer, 1778), also known as the chicken mite or red mite, is a common mite species worldwide. This mite, which is seen in laying hens, especially in free range farming, can also be a problem in caged systems. PRM is an obligate blood-sucking parasite that is dependent on the host, and causes many health problems in birds, especially anemia and irritation, and it adversely affects animal welfare in different aspects. It is thought that the improvement of the breeding systems will actually be in favor of the PRM. Because each piece of equipment in the poultry houses means more hiding and breeding places for PRM. This will increase the number of control and treatment points in the henhouse and cause the struggle to become more difficult. Konyalı and Savaş (2021) reported that there is a positive relationship between the improvement of henhouse conditions and mite density. The same authors reported that the prevalence of mites was higher in well-ventilated henhouses having perches (Konyalı and Savaş, 2021). On the other hand, it was observed that the prevalence of mites was higher in other systems such as free range or organic farming systems compared to cage-raising systems (Sparagano et al., 2009). Dry litter is a good hiding place for mites. Especially dry feces covered with straw or sawdust are the main areas where mites nest and reproduce. In addition, enriched cage systems are the main places for the survival and nesting of the mites, especially when they contain equipment such as nests or perches. Chirico and Tausan (2002) reported that although enriched cage systems contribute to improving animal welfare, they could unfortunately also optimize the survival conditions of mites. In this sense, the change of the cage breeding system current days increases the risk of mites due to both more equipment and the litter situation of breeding on the ground. The automatic litter cleaning system is allowed to remove the mites in the caged systems. But this opportunity will decrease in free range systems. In addition, it means that if the animals go to the free-range areas, the mite moves to more areas inside or outside of the henhouses. It was observed that the litter piles stowed near the shelter contain a large number of mites. If there is a manure pile accumulating close to the barn, this will mean that the mites have been moved back into the shelter. It should not be forgotten that even if hygiene and spraying studies are carried out inside the shelter, there will be mite transfer from this manure to the poultry house.

It is possible that the prevalence and activity of PRM will increase and cause more problems over the years' parallel improvement of hen housing conditions. This situation creates the need for update the current approaches to control PRM. Sparagano et al. (2020) reported that more than 400 million laying hens in Europe were infested with mites with a prevalence ranging from 60-90%, with an estimated impact on the egg industry of €231 million per year. However, it should be noted that this cost is much more harmful than the estimated amount considering due to its indirect effects and other poultry production systems.

The fact that the PRM being active at night negatively affects the host's struggle with them. In addition, another weak point in the individual host's in struggling against mites is beak trimming applied in the poultry industry. The ability of the hen to take an adult mite, which is about 1 mm in size, decreases after beak trimming. Many European countries in are banned to beak trimming within the scope of animal welfare regulations. On the other hand, it is not known what the consequences will be in case of high mite infestation, which is positive for animal welfare. Because, the main and first effect of the mite on the host is itching. It is possible that this mite-induced itching and stress may promote aggressive pecking in non-beak trimmed animals (Sparagano et al., 2020).

DERMANYSSUS GALLINAE IN POULTRY

Dermanyssus gallinae is a hematophagus mite that feeds on blood, that is dependent on its host. It is the most the external parasite which affects the poultry industry almost all over the world.

The reason is why very important ectoparasite that it is seen even in poultry breeding systems that are completely controlled. Although its main host is birds, it has been reported that it can attack other mammals, including humans (Sikes & Chamberlain, 1954; Duncan 1957; Hoffman 1987; Haag-Wackernagel, 2008). It has 5 life cycle that starts with the egg and the subsequent larval, protonymph, deutonymph and adult stage. After the larval stage, it begins to feed on blood at the protonymph stage. After the molting stage that follows, it passes into the deutonymph stage and then becomes adult by being fed with blood. Knowing these stages and biology of the poultry red mite is especially important in terms of development of control methods. Because the effectiveness of some applications, including chemical application, is changed according to these life stages. In addition, the visual detections could fail in the first 3 life stages inside the henhouse. The monitoring and early detection is most important point in the control of poultry red mites. Therefore, detection of nymphs and larvae is possible only by knowing these stages.

Dermanyssus gallinae has a high potential reproductive ability when begins to reproduce with the transition to the adult stage. An adult and mating mite lays between 1 and 9 eggs every 12 to 24 hours. A female mite can produce around 30 eggs on average within a few blood-sucking and ovulation cycles in her lifetime (Huber et al., 2011). A mite matures in just 7 days under normal conditions, for this reason high infestation can occur in a very short time. The best favorable temperatures for juvenile development and egg laying are between 25 and 37°C, (Maurer & Baumgärtner, 1992), and the relative humidity for best survival conditions is between 70-90% (Nordenfors et al., 1999). PRM could survive up to 9 months without feeding under moderate temperatures circumstances (5-25°C; Nordenfors et al., 1999). PRM has many harmful effects on the poultry sector because of this survival and reproduce characteristics. Its effects on the host are not only blood-sucking, but also PRM has direct or indirect effects on host, farms and public health.

The main effects of PRM, which is a hematophagous ectoparasite, on the host are blood loss and anemia on the health of the host. Depending on the mite burden and the immunological ability of the host, various health problems arise that may result in the death of the host. Konyalı and Savaş (2016) reported that environmental factors such as breeding and feeding conditions may also be effective in the emergence of the harmful effects of PRM, and same authors stated that regenerative anemia can be seen under optimum environmental conditions. In this context mite burden, mite exposure time, immunological structure of the host, breeding and feeding conditions are the factors that determine whether these effects occur or not, and constitute individual variation in terms of resistance.

The effects of PRM differ according to mite load, host biology and environmental factors. These effects on host health are summarized below.

- Anemia and deterioration of other hematological values,
- Inflammation of the skin and irritation,
- Stress,
- Deterioration of immunity,
- Acting as a vector,
- Behavior problems,
- Asymmetry,
- Deterioration of reproduction.

PRM not only affects the health of the host, but also it has negatively effects on the yield parameters. These yield parameters decreasing due to infestation are;

- Body weight
- Growth rate,
- Egg yield and quality,
- Feed efficiency,
- Meat yield and quality,
- Delay of sexual maturity,
- Delay of first laying age and 50% yield age,

- Chick quality.

The reason why the poultry red mite is so important is that it affects performance that are not immediately visible or quantifiable. For example, the decrease of feed efficiency, decline in growth rate and body weight gain, delayed sexual maturity are some of them. The most obvious effects are the decrease in egg and meat yield and quality. All these factors affecting efficiency, of course, also affect the profitability of farms. The other effects on farms are the treatment costs and labor forces against control of these parasites. Discharge of henhouses, disassembly and assembly of shelter equipment, spraying of henhouses and equipment, and all kinds of cleaning and disinfection works mean more labor and energy consumption in farms. Ünbaş et al. (2020) reported that the chicks of infested parents had lower Tona score and higher respiratory rates than uninfested parents. In that context, the aforementioned authors concluded that breeder flocks infested with PRM could havedetrimental effects on the hatching traits and chick quality (Ünbaş et al., 2020).

Effects of PRM on farms are summarized below;

- Decrease of profitability,
- More labor,
- Increase of costs,

PRM also poses risks in terms of public health. Poultry farm employees are in the first risk group. PRM causes itching, allergies and dermatitis in humans. Although it is reported that it is difficult to estimate the costs arising from *D. gallinae* in poultry farms, it has been reported that farmers in some countries pay their workers up to 3 times more than those working in flocks that are not infested with *D. gallinae* (Sahibi et al., 2008). In addition, the nests of birds near the public such as pigeons and swallows pose a potential risk. These effects on public health are;

- Itching, allergies and dermatitis in humans,
- Stress and discomfort,
- Residual risks,
- Infestation risk of pets.

THE USE INTEGRATED PEST MANAGEMENT TO CONTROL OF POULTRY RED MITE

Different methods have been tried for many years in the control against parasites. The first methods against to external parasites, which were an important problem at every stage of agricultural activities, started with the use of sulfur by the Sumerians. The Egyptians and the Chinese used herbs and oils to control of pests, and then different civilizations tried different methods. Especially in Europe, as the years passed, new inventions brought new applications, and new problems and needs emerged in the Renaissance and later periods. Every innovation made to increase productivity has caused another problem. Observing the toxic effects of substances such as arsenic and DDT, which are used as a solution, has also led to a ban. It has been emerged that the unconscious and excessive use of chemicals caused some problems such as resistance of parasites, target pest resurgence, and secondary pest invasion. In addition, the chemicals used with the detection of bioaccumulation caused accumulation in other living things and humans. As a result of this, the use of some active substances is prohibited in some countries.

The necessity of the IPM in the control of the PRM has emerged at several points. The first of these is the resistance developed by mites against synthetic chemicals and the failure of chemicals used after a certain period of time. In addition, applications made without knowing the biology and behavior of the mite result in failure. The reasons such as the seasonality of the mite or the change of breeding and feeding behaviors of mite according to environmental conditions require acting with the IPM. The synthetic chemicals cause harmful effects not only on animal products but also on animals, including allergic reactions and death in overuse. In addition to all these, the increase in labor and application costs requires alternative methods in the control against to mites.

The main purpose of IPM is to use appropriate management and techniques to keep the pest population below a certain harm threshold. Each technique must be compatible with the production goals and means.

The temperature and relative humidity are the most important factors in the reproduction of the mite in the presence of a host. It was reported that the highest rate of reproduction and the least mortality were observed between 20 and 37 °C (Maurer and Baumgärtner, 1992; Nordenfors et al., 1999). In these climatological conditions, the mite population increases very rapidly and a henhouse can be heavily infested in a short period of time (Maurer & Baumgärtner, 1992). Therefore, early detection is important.

One of the basic elements in integrated pest management is the prevention of pest transmission. It is difficult to control external parasites, especially *Dermanyssus gallinae*, which can spread to the entire henhouse and not stay on the host. The first condition of combating *Dermanyssus gallinae* is to know the transmission and spread routes and to take the necessary precautions. All kinds of equipment and live animal exchanges between henhouses can cause the transfer of adult mites, nymphs or parasite eggs. Eggs, chicks or pullets taken from an infested poultry house are important transmission and transport routes for the spread of PRM. In addition, human movements between henhouses can also cause mites to be transported. Mites that climb on people's shoes, clothes or certain parts of their body can easily be moved to other coops and these mites can give rise to infestation in the progress of time. In addition, feed and similar products and equipment such as sacks are potential transportation routes. Litter and manure thrown from infested coops can also contain mites intensively. One of the biggest mistakes made is to throw these manures near the cleaned houses. It should not be forgotten that these manures are the most suitable habitat for mites, and these removed litter must be kept away from the henhouse. Because the mites found in these litter/manure heaps can easily infest the henhouses again.

The feathers of infested animals are also potential transport routes, and their movement also allows the mite to be carried from one place to another. Wild birds, mice and other animals facilitate the transport of mites, especially the bird nests near the henhouses pose a significant danger. Swallow, sparrow or pigeon nests, which are frequently found in residential areas, pose a risk (Auger et al., 1979; Bellenger et al., 2008; Haag-Wackernagel, 2008), such as PRM was also detected in the nests of swallows that nest on the edges of the windows of the houses or in living areas such as balconies, and the mites found in the nests enter the house through the window openings (Konyalı and Savaş, 2016).

Another key element of the integrated pest management is early detection and timely treatment. In this context, the first step is to detect the presence of the mite as early as possible. Generally, the mite density increases with the warming of the weather, that is, starting from the spring. However, the presence of mites continues almost throughout the year in environmentally controlled poultry houses. Therefore, periodic monitoring of henhouse and animals is necessary for early detection. Because of this feature of PRM, which is a mobile external parasite, mite aggregations are seen at very different points in the henhouse. As a result of the late detection of these clustering points, the mite density will inevitably increase. Therefore, the first question of farmers is where the mite is found. The equipments, especially made of wood, are the places most preferred by the mites.

The possible aggregation areas of PRM in the henhouse are summarized below.

- Litter (especially dry litter)
- Feeder, drinker and related equipments
- Cracks and crevices in walls
- Beams, posts and other structures
- Perches and nests
- Joints of window and ventilation equipments
- Any other equipment and structure in the poultry house
- Outdoor ranging area

There is a visible color difference between a fed mite and an unfed mite and the color of a fed mite can vary from red to dark red or even black, while an unfed adult mite can be a pale color close to light brown to off-white (Konyalı and Savaş, 2016). It is useful to take this situation into consideration when scanning for mites in the henhouses. Because the detection of small and hidden mites becomes difficult due to their color, especially if they are not fed.

Another method for monitoring and follow-up of the PRM is the use of traps. Different types of traps can be used (Kirkwood 1963; Nordenfors et al. 1999; Mul et al. 2015; Konyali 2016; Mul et al., 2017). The aim is to create an aggregation area where the mite can reproduce and shelter, and to select the optimum treatment time for the population present there. It is important for success that choosing a trap suitable for the henhouse and breeding system, and determining the place in the poultry house. There are some issues that need to be considered in the process of tracking using traps. First of all, the traps must be at places where they will not get wet in the henhouse and it is necessary to periodically check whether the traps are damaged. Traps should be placed at more than one point in the henhouse, especially perches and nests. In the cage system, especially the bottom and part of the cage bars are ideal for placing traps. Mul et al. (2017) reported that the DAP model, which will help predict mite population dynamics, can be used in the management of IPM for this purpose. The use of appropriate traps is important for early intervention, especially in temperature-related population increases.

It should not be forgotten that due to its reproductive cycle, the presence of the population can turn into a heavy infestation in a short time, and the intensity of infestation may be much greater than it seems. If people are infected and nymphs are seen wandering around when entering the henhouse, this is an indication of high infestation. If this parasite also appears during the day, it indicates heavy infestation.

Determination of the most appropriate time for treatment to control of PRM is one of the most important issues. Host behavior can often be a good indicator in addition to trap use and poultry house monitoring. Konyali et al. (2018) reported that infested animals exhibit 3.74 times more scratching/preening behavior and move 2.5 times more than uninfested animals. The PRM has piercing-sucking mouth structure, so this mouth structure and feeding strategy causes animals to be more active and restless. Stress occurs in the host, which is exposed to mites, especially at night, that is, during the resting period. It is possible that this mite-induced itching and stress may promote aggressive pecking in non-beak trimmed animals (Sparagano et al., 2020).

It is important to know the spread and transport routes for effective control strategies against PRM. In this context, the ways in which the PRM can be transported and infested are summarized below.

- Equipment such as nest bowls and drinkers,
- Egg and viols,
- Feathers that fly or move,
- Bird, mouse and other animals,
- Human,
- Feed materials and similar products,
- Chick or pullet,
- Feces, manure, litter.

There is more than one answer to the question of where the mite can cluster in the poultry house, which may vary according to the conditions. Namely, as a result of our research and observations, PRM;

- Prefers areas where are slightly cooler than the temperature is high inside the henhouse,
- Prefers areas where have better ventilation if ammonia is high or the environment is airless
- Prefers areas where are dry if the ground is wet
- Prefers areas where that have not been sprayed if spraying has been done with synthetic chemicals. It has been observed that they climb away from the ground or equipment applied to the acaricides and cluster in chemical-free places such as the upper points of the henhouse.

This motility of the PRM is very important for the surviving mites to reaggregate and reproduce and to maintain its existence.

Reasons are summarized below why traditional methods are ineffective.

- Residue problem in animal products as a result of the use of synthetic chemicals
- Genetic resistance acquired by the parasite as a result of the use of synthetic chemicals
- Not using the correct active ingredient
- Errors in application time and frequency
- Errors in application places

One of the reasons that reveal the importance of integrated pest management is the loss of effectiveness of the traditional methods used. There are multiple reasons for the ineffectiveness of traditional methods or criticism of their use, all of which are interrelated. In other words, the most important reason to necessitate to alternative methods is the interaction between applications. Primarily, one of the results of unconscious and excessive synthetic chemical application is the resistance developed by parasites against these chemicals. As a result, the success rate in subsequent applications is decreasing day by day. As a result of long-term application of acaricides, it has been reported that mites develop resistance to these acaricides (Marangi et al., 2012; Abbas et al., 2014). This situation negatively affects the effectiveness of acaricides. Zdydbel et al. (2011) also stated in their study in Poland that the effectiveness of the acaricides used in a similar way differs according to the regions, and this is due to the development of resistance by the mites. Therefore, it would be more appropriate to abandon the unconscious and excessive use of acaricide and then to use a control program for poultry red mite control.

There is an example protocol for controlling PRM, that is, keeping the infestation density below a certain threshold. As mentioned above, the first purpose is to prevent infestation. Therefore, new chicks, pullets or hens must be obtained from mite-free farms. In addition, the equipment or people should be allowed to enter the henhouse by paying attention to the hygiene conditions and making the necessary controls.

It can be said that the most important stage of the protocol is mite control and monitoring. Because early detection is very important in terms of preventing infestation density. Therefore, as mentioned before, traps should be placed at certain points of the house and periodically checked. Simultaneously, other points of the henhouse must be constantly checked. It is necessary to know where the PRM can be hidden in the henhouse and to make the controls at those points. As mentioned above, dry litter, perches close to the host, points such as mangers and any cracks, crevices or equipment joints are the clustering points of PRM. The effect of the season on the increase in the infestation density of PRM should not be ignored. Therefore, especially the periods when temperatures start to increase, such as spring, are suitable breeding and reproduction times for mites. It should not be forgotten that environmental conditions, namely temperature, have a significant effect on becoming adult after spending the winter or adverse conditions as eggs. In other words, even if mites are not seen in the henhouses in some periods, one of the reasons for the subsequent infestation is their high ability to withstand adverse environmental conditions (especially low temperatures) as eggs, nymphs or adults.

The first thing to do after observing the mite that is the mechanical cleaning of the house before spraying. Otherwise, the chemical applications to be carried out may not affect the mites at the desired level. For this, the feces, including the manure, must be cleaned and scraped in the poultry house.

It should be noted that litter shed or kept near the poultry house contains a large amount of mites. Therefore, the cleaning process and its aftermath should be managed correctly. Cleaning should not be limited to only the litter, but also equipment such as perches, nest bowls and drinkers should be cleaned. Liming after cleaning can be a priority application for low infestation. It is known that ash has been used for years in against mites in back yards. In fact, the aim is to damage the cuticle of the mite and to death of mite due to dehydration. Silica-based powder acaricides are commercially available and they have similar effect to ash. Humidity conditions are also important in the effectiveness of these powders, and it is reported that success decreases at 85% and above humidity values (Harrington et al., 2011). It is necessary to know that these applications do not eliminate the mite 100%. Therefore, mite monitoring should be continuous throughout the year. If the mite increase is high, re-application is required after detailed cleaning. For this purpose, synthetic or plant-based pesticides can be used but it is necessary to pay attention to the necessity and application protocol. Due to the residue risk, the choice of active ingredient varies according to the countries. Therefore, it is necessary to know that every synthetic pesticide should not be used during the production season.

Some farms go to the path of applying synthetic pesticides after removing all the animals from the poultry house, which means a serious labor force. According to the IPM strategy, the use of non-chemical acaricides should be the first method of application. The essential oils that bay, cade, cinnamon, clove bud, coriander, horseradish, lime, mustard, pennyroyal, pimento berry, spearmint, red thyme and white thyme were effective on *Dermanyssus gallinae* (Kim et al., 2004). Additionally, neem tree, tea tree and garlic oils were had toxic effect on *Dermanyssus gallinae* (Choi et al., 2004; George et al., 2009). Magdaş et al. (2010) reported that oils of sweet basil, coriander, peppermint and Summer savory were the most effective against PRM. It is not recommended to use the same active ingredient continuously in synthetic pesticide applications due to the possibility of resistance of mites. Therefore, pesticides with different active ingredients can be used alternately. In addition, it is necessary to re-application within 1 week after the application. Because, after a certain period of time, mites may be seen again in the henhouse, both for the surviving mites and for reasons such as the pesticide not reaching the eggs or not being able to affect them. To prevent this, reapplication is required after 1 week.

Table 6: Sample Control Protocol for *Dermanyssus gallinae* in Poultry Houses.

Phases	Objective	Control Points	Check List
Phase 1	Control of mite transmission routes to the poultry house	Pullets or hens from infested poultry houses present a potential risk.	Information about the external parasite history of the breeding henhouses
		Wild animals	Bird, mouse etc.
		Staff and guest entries	Disposable clothes
Phase 2	Detection and monitoring of mite presence	Trapping	During all year
		Control of aggregation places inside the henhouse	Attention to seasonality
		Control of animal behavior	Especially itching/preening
Phase 3	Mechanical cleaning	Litter stripping and cleaning, liming	Periodically - More often in case of high infestation
Phase 4	Trap and henhouse monitoring	Acaricide application in parallel with the increase of mite infestation	Periodically - More often in case of increase in mite infestation
Phase 5	Treatment of acaricide after cleaning	Application points are important	Care should be taken to use appropriate active ingredients that will not pose a residual risk.
Phase 6	Repeat acaricide treatment	Application of an acaricide with different active ingredient after 7 days	Application sites are important
Phase 7	Attention to equipments	Dismantling and spraying of equipment, if necessary, in heavy infestation	Attention to perches and nests
Phase 8	Trap and henhouse monitoring	Traps that are dirty, worn out or already nested should be replaced with a new one.	During all year

The use of standard control strategies could be difficult due to the success of treatment varies depending on several factors. Mul et al. (2017) is summarized these factors as temperature, humidity levels, husbandry systems and breed. Creating a biosecurity checklist suitable will be an important contribution on PRM control.

CONCLUSION

As can be seen, poultry management is the main factor in the control of mites. Improving breeding systems on the basis of animal welfare actually increases the survival likelihood of mites in these systems. In addition to the fact that the methods used to control of mites are still not 100% effective, the problems brought about by chemical applications have required alternative control methods. However, the point to be considered here is the necessity of not only one method, but also more than one method. In this sense, IPM is an important control of poultry red mite. Thanks to the control protocols to be created on the basis of this IPM, the *Dermanyssys gallinae* infestation density in the poultry houses will be kept below a certain threshold. It is important to include the control of poultry red mite in poultry farming within the framework of biosecurity measures in order to keep the prevalence of mites under control levels at the regional or national level.

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CHAPTER 12

CURVE FITTING IN AGRONOMY: PROSPECTS AND CAUTIONS

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INTRODUCTION

Improving our understanding of plant growth and development is a principal consideration in agronomy. Evaluating the growth pattern of plants using non-linear regression models is a practical and effective approach to identify their growth. Whether it is a plant, animal or a bacterium, growth patterns in biology are generally consistent with each other: Growth is initiated slowly, which is followed by a period where growth rate increases until the organism reaches to its maximum growth capacity. This increase of growth rate is not continuous: after reaching to its maximum value, growth rate gradually decreases and eventually reaches to zero. After this point, some characteristics (plant traits such as dry weight or plant height) may preserve their value when others may reduce. Regardless of the conclusion, entire process of biological growth resembles a stretched version of "S" word, which is why a certain class of non-linear regression models, the sigmoidal models, are utilized widely on growth analysis. In addition to their good compatibility, increasing popularity of sigmoidal models in agronomy is also a result of their wide adaptability and usefulness in terms of enabling meaningful interpretations.

This chapter is dedicated to the utilization of sigmoidal regression models in agronomy, including a brief summary of their developments, current capabilities and limitations. In order to execute a meaningful growth analysis, understanding the basics of the regression analysis is not enough: a general knowledge about the use of individual models is also a requirement. Challenge of an agronomist is to decide which models to use and how to interpret the parameters and critical points of the curves. This calls for a wider point of view beyond the goodness of fit and includes several key factors such as model convergence. Hence, aim of this chapter is to present an overview about important issues about curve fitting concerning agronomy.

CURVE FITTING IN AGRICULTURE

In statistical terms, curve fitting adjusts a nonlinear regression model to fit the growth data best according to the algorithms such as Marquart-Levenberg or Gauss – Newton. Flexibility of non-linear models is probably the most important factor explaining the popularity of curve fitting. A regression model with one parameter (such as linear regression) can be considered more rigid compared to other regression models with two (such as quadratic regression) or three parameters. More parameters on a regression equation indicates “curviness” and increases the flexibility (Motulsky & Ransnas, 1987) by allowing it to fit better to a wider set of data. Given the number of model parameters and different parameterizations of their equations, nonlinear regression family contains highly adaptable models which have been intensively utilized in scientific literature. Use of growth curves on growth data has several advantages compared to ANOVA: as stated by Sari et al. (2019a), regular harvests from an experimental unit can’t be randomized, which is an important requirement for ANOVA. Curve fitting also does not require normality or homoscedasticity of residuals and could even be used for hypothesis testing (Sari et al., 2019b). More importantly, fitting growth curves on a growth data provides an assessment regarding the entire growth trend, therefore provides a different point of view that leads to other meaningful interpretations.

Growth analysis with non-linear models have been utilized for a wide range of uses in many areas. For example, the Gompertz sigmoidal growth model, which is now a well-known growth model in biological sciences, were first developed by a mathematician named Benjamin Gompertz in 1825 to identify his theory of human mortality (Winsor, 1932). Curve fitting is also a useful tool within the social sciences in cases when a longitudinal data (data/measurements are collected from the same group of individuals for a period) is analyzed (Curran et al., 2010).

Historically, reports indicate that the use of growth models in agronomy and animal science has been continuously increasing and diversifying. First reports of their use are dated back to the mid-19th century, although it is impossible to know exactly when and how they were developed. Using growth curves in agronomy seems to gain popularity among researchers by 1990’s. According to De Reffye et. al. (1997), the classical approach of curve fitting is “ecophysiological”, where the changes of dry matter production of plants (and by some extend, plant parts such as leaves, stems and roots) under different environmental conditions were predicted by growth models. Additionally, aim of the study could also be limited to the comparison of plant varieties or species. Many studies from this era also identified the best fitting models for their experiments as a suggestion for future studies in addition to the conclusion of curve fitting being a useful method to evaluate growth. Following this period, use of sigmoidal growth curves were increased in both agronomy and animal production.

A short list of several important sigmoidal models is given in Table 1 (Panik, 2014). It should be noted that there are many different equations regarding to these models: presented models have many different derivations as a natural result of their adaptability (Tjørve, 2003; Karadavut et al., 2017; Miguez et al., 2018). Therefore, agronomists who aim to utilize these methods in their studies today can benefit from a massive literature both within and outside of their study areas. Several statistical/mathematical reviews about the curve fitting (Kvam & Vidakovic, 2007; Efromovich, 2008) could be helpful for those who understands the basis of the theory and wishes to expand their knowledge. Additionally, Motulsky & Ransnas (1987), Archontoulis & Miguez (2015) and Panik (2014) provides a simpler but informative introduction to the theory and application of many nonlinear models.

Table 1: Several important sigmoidal growth models used in agricultural sciences

Model	Equation
Logistic	$Y = \frac{a}{1 + be^{-cx}}$
Gompertz	$Y = ae^{-be^{-cx}}$
Weibull	$Y = a - be^{-cx^d}$
Richards	$Y = \frac{a}{(1 + be^{-cx})^{1/d}}$
Von Bertalanffy	$Y = a\left[(1 - be^{-d(1-c)x})\right]^{1/1-c}$
Morgan Mercer Flodin (MMF)	$Y = \frac{ab + cx^d}{b + x^d}$
Explanation: The letter *e* represents the mathematical constant, *x* represents time and *a, b, c* and *d* are curve parameters. Curve parameters can also be named with latin words (α, β … etc.) or consecutive numbers (Parameter 1, 2 ... etc.).	

In this chapter, I aim to introduce their use in agronomy with an emphasis on model convergence to provide a practical point of view. Having that said, I strongly recommend these publications for a wider perspective.

General properties of plant growth curve analysis can be summarized as follows:

1) **Growth data consists of plant measurements taken in equal intervals.** Length of the intervals are usually days or days after

sowing, but it can be weeks, growth degree days or different stages of growth, depending on the aim of the study.

2) **Typically, a reliable indicator of plant growth and development is measured over time.** Majority of agronomic studies prefer to evaluate dry matter accumulations. Using fresh weight is usually avoided since the varying moisture level of consecutive samplings can increase experimental error. Plant height can also be used but evaluating the dry matter accumulation of a plant provides more meaningful interpretation – and a better approximation of growth (Demotes-Mainard & Jeuffroy 2004; Giri et al., 2019).
3) **Selecting Models.** Model selection in curve fitting is a critical issue. Firstly, shape and general distribution of growth data should be evaluated with a basic two-way graph where growth data is fitted against the sampling dates. This evaluation should give an idea about which models can be appropriate, however evaluation may not be a simple task since there are many nonlinear growth models (sigmoidal or others) to consider. Ultimately, a researcher should have a general idea about which models to choose before beginning the analysis, for which a preliminary review of literature would be helpful. In addition, a researcher should also be familiar with the models before using them since using a wrong model will not lead to an useful interpretation, even if the model appropriately fits the data (Sit & Costello, 1994). Only after these careful processes, a preliminary analysis including several models would reveal the goodness of fits of each model, allowing for a selection on a statistical basis.
4) **Goodness of fit. "**Goodness of fit (GOF)" means how well a chosen statistical model corresponds to the data. In other words, a higher GOF indicates a lower error justifying the convenience of the model. In curve fitting, the most popular GOF statistics are coefficient of determination (r^2), standard error (SE), corrected Bayesian information criterion (BICC) and corrected Akaike information criterion (AICC). A better GOF is where r^2 is as close to 1 as possible when SE, AICC and BICC are minimum, indicating that deviations between the predicted model and actual data is minimal. Curve fitting usually yields high GOF (usually above 0,9 r^2) if growth data is

collected properly and a suitable model is selected. This is due to the iteration procedure: since curve fitting is the process of creating a curve that fits the data points best, curves go through a series of iterations (may be several iterations or hundreds, depending on the data, model, and the software) until the best fit is achieved. Iteration process ends only when algorithm can no longer increase the GOF– thus concludes that the best possible approximation of the data is achieved (for more information, please see the discussion above about the convergence). Therefore, in general terms, low GOF can be the result of choosing an inappropriate model or inconsistencies among the growth data (outliers etc.). In most cases, viewing the curve and the data on the same graph should provide an insight about what went wrong. Additionally, checking the residuals is also recommended.

5) **Checking autocorrelation.** In addition to GOF, chosen model should also be tested for autocorrelation. Durbin – Watson (DW) test is one of the most popular autocorrelation tests in the curve fitting. It tests whether the adjacent errors of the regression fit are correlated with each other, which would mean positive or negative autocorrelations. In cases where autocorrelation is detected, results of the curve fitting is considered doubtful. Other commonly used autocorrelation tests are Shapiro-Wilk and Breusch-Pagan.
6) **Results.** Curve fitting with growth models identifies the plant growth in statistical terms. This approach yields model equation containing data specific parameters. For example, a three parameter Logistic model is fitted to the weekly observations of oat height (Figure 1) using Curve Expert Professional v. 2.7.3 software (Hyams, 2010). Resulting equation is a logistic equation where *a*, *b* and *c* parameters are specifically calculated to fit the data. Obtaining this model equation is the ultimate output of the curve fitting. All interpretations are directly or indirectly based on these specific model parameters. In some cases, these model parameters can be evaluated directly (such as parameter *a*) or indirectly by calculating critical points of the curves (please see the interpretations section). Different calculations of growth rates can also be evaluated (Paine et al., 2012). Moreover, statistical significance between genotypes or applications can be checked by comparing the curve parameter of one to the confidence interval of the other's, which would reveal whether an application

causes statistically significant differences on growth curves (Carini et. al., 2020).

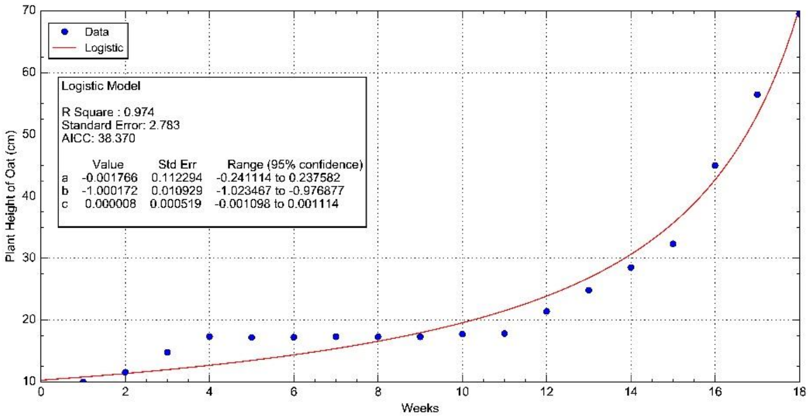

Figure 1: Explaining plant height increase of winter oat with Logistic Model

7) **Growth model recommendations.** Many earlier research aims to compare the goodness of fit parameters of several growth models to reveal the best models for their data.

8) **Interpretations.** In agronomy, fitting a curve on a growth data leads to many interpretations. It gives precious insights about critical times on plant growth for a more precise agricultural management (Karadavut, 2009; Hocaoglu & Coşkun, 2018) while it also generates predictions. Additionally, curve parameters can also be interpreted, allowing the comparison of genotypes or species. A practical review about evaluating how model parameters define the shape of a curve is presented by Tjørve (2003). According to this review, parameter *a* (the first parameter) of sigmoidal models represents the upper asymptote. This is the reason why nearly all studies containing a growth curve analysis with a sigmoidal growth model includes the interpretation of the parameter *a*. Since the upper asymptote of a sigmoidal model is essentially its theoretical maximum point, parameter *a* is a good indicator of maximum growth. Other curve parameters are also mentioned in several studies, but they are not as often interpreted as the first parameter. There are several reasons for this: their roles are not as specific and may vary according to the

selected model. Parameter *b*, for example, is associated to the abscissa of the inflection point of Logistic and Gompertz models, but there is an ongoing discussion about whether this parameter can be interpreted biologically (Fernandez et. al., 2015). It should be mentioned that in general, curve parameters define the shape of a curve collectively. Thus, they are rarely interpreted as their own. Some sigmoidal growth curves have critical points, which allows for further evaluations. The most renown critical point of a sigmoidal growth curve is called “the point of inflection (PI)” a.k.a. “the inflection point”. PI is the point on the curve where growth rate reaches to its maximum which comes before the actual point where observed plant / animal reaches to its maximum growth. PI is calculated on curve parameters for all models, but its equation differs (Goshu & Koya, 2013); hence the PI of different models will be slightly different from each other even if the same data is used. Interpreting PI varies according to the aim of the study. In general, lower PI values indicates an earlier growth (Sari et al., 2019a Hocaoğlu, 2022). In addition to PI, there are several other critical points associated with other critical changes of growth rate. Their calculations allow for further biological interpretations. Mischan et al. (2011) identified these on Logistic curve where: 1) growth rate increase is maximum, 2) growth rate reaches to its maximum (IP), 3) growth rate decrease gets maximum and lastly, 4) growth rate reaches zero (maturity). This approach has highly improved the usefulness of curve fitting in biological studies. Several other studies following Mischan et al. (2011) adapted these points to compare the harvest maturity of tomato cultivars (Sari et al., 2019b), strawberry cultivars (Diel et al., 2022) and optimize the harvest time of lettuce (Li et al., 2022).

CONVERGENCE

Even though the goodness of the fit parameters indicates the success of the curve fitting, they are not the only measure for its usefulness. Projections generated by the sigmoidal models may not always be accurate despite having an adequately high R^2, a low AICC and low standard errors. Another important measure about curve fitting is “the convergence”, which is a term used in nonlinear regression analysis, suggesting the overlapping of model predictions to the data (Dasgupta, 2013). Ideally, a model with high GOF can be expected to converge to data well but this may not always be the case when a chosen model is simply inappropriate for the given data. Model convergence can be observed on the change of residuals during the iteration processes of curve fitting (Motulsky & Ransnas, 1987). An example of this process can be seen in Figures 1, 2 and 3 reflecting the iteration process of the curve presented in Figure 1. Model parameters (usually named with letters such as *a*, *b* and *c*) goes through a number of iterations in order to decrease the difference between the projections of the curve and data points (Figure 2). These differences are called *residuals* and lesser residuals indicate a better fit (Figure 3). Iterations ends for two reasons: First, the parameters may reach their final values where they converge with the data – hence the curve is (more or less) adjusted to it. Alternatively, iteration process may be over because the maximum number of iterations (which can be hundreds) are reached but the model convergence hasn’t improved. In this case, residuals will not seem to reduce in response to the iterations. This simply means that some sigmoidal models may occasionally fail to explain certain data while others succeed. These curves might still have a good fit (the goodness of fit parameters are the indicators of that) but using their parameters requires caution. In the case of an oat plant height curve presented in Figure 1, plant height increase of oat was successfully modeled with Logistic regression. Success of this curve fitting depends on several observations: Firstly, the GOF parameters indicated a good fit. Additionally, model parameters seemed to settle during iteration process (Figure 2) while as the residuals continued to decrease until the end of the iterations (Figure 3). Here, it should be mentioned that iterations ended before the maximum number of iterations reached (in this case, maximum number of iterations were 100), which means that the iteration process ended because model parameters were no longer changing – thus, the algorithm concluded that the curve reached its “best” fit possible (Archontoulis & Miguez 2014 Hyams, 2020).

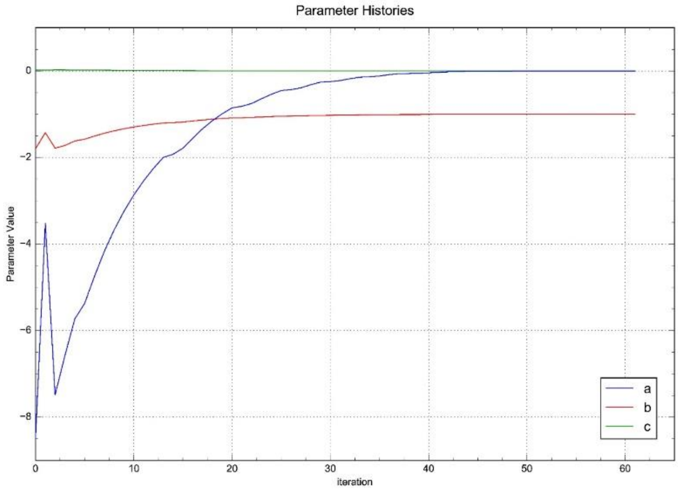

Figure 2: Parameter changes during iteration process

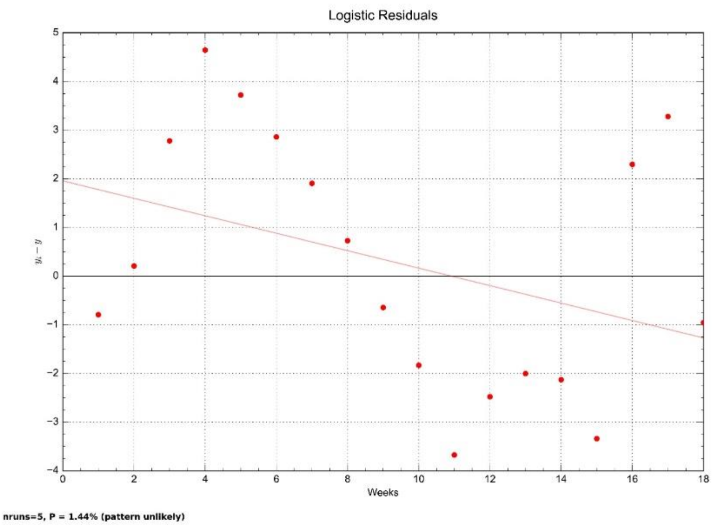

Figure 3: Residuals of each sampling

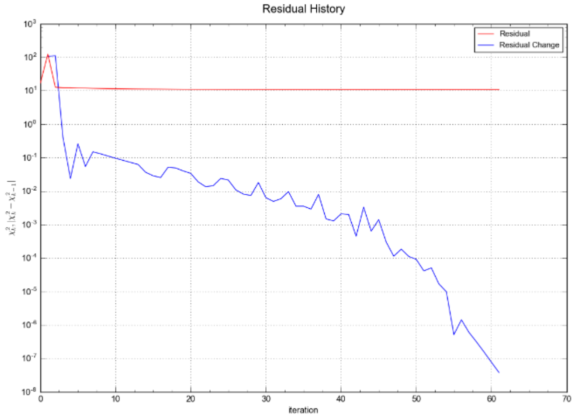

Figure 4: Change of residuals due to iterations

In addition to the convergence, model predictions can also provide critical information about whether the chosen model is inherently suitable to express the nature of the given data. An example of this can be seen in the curve fitting on Figure 5 where Logistic Power, Gompertz and Ratkowsky models were fitted on dry matter increase of barley revealing the differences of their upper asymptotes (data obtained from Hocaoglu et. al., 2022). Despite their good fit, examining the future projections of each model on the same exact graph reveals the importance of the last few samplings and their effects on the curve fitting. The goodness of fit parameters of all these models indicated an excellent fit ($r^2 > 0.98$), but Gompertz model falsely forecasted a substantial increase in the future, causing inconsistently high parameter *a* since it is associated to the upper asymptote. On the other hand, Ratkowsky model (which is another popular sigmoidal growth model) seems to explain the nature of this data better. Thus, I recommend using multiple sigmoidal models and taking their parameter values into account in addition to the known measures of the goodness of fit and the convergence.

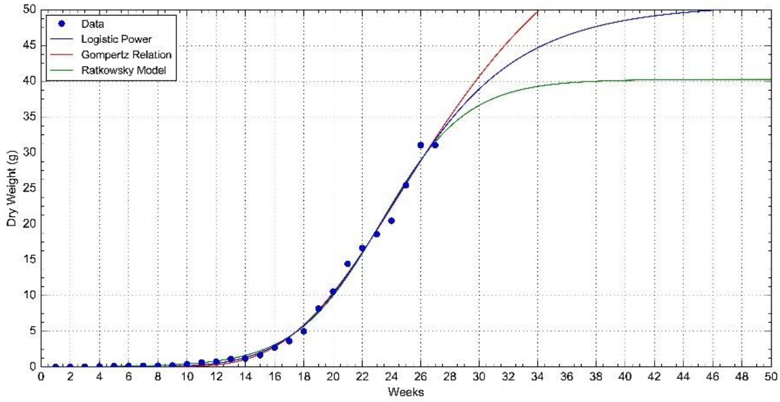

Figure 5: Extended view of the Logistic Power, Gompertz and Ratkowsky curves on dry matter increase of an oat genotype.

FUTURE REMARKS & CONCLUSIONS

The history of plant modelling is also a history of consecutive advancements. The fact that all models, evaluation methods and their implications were derived from the elementary mathematical equations of 19th century is extraordinary. In terms of individual assessments of plant biology, we can identify the growth, obtain accurate predictions, and pinpoint critical turning points. This paves the way for a whole different line of studies contributing to the literature of plant and animal biology. This approach can be utilized in many subjects: from genotype/species comparisons to the factors affecting the growth, such as stress factors or applications. Due to this potential, there have been a great deal increase in the use of curve fitting recently.

In addition to these uses, fitting regression curves on growth data is only a part of a bigger vision. Methods used in modelling plant growth and development has already been incorporated into bigger and more sophisticated algorithms. Today, there are several comprehensive plant models used on a field scale which can simulate important parameters such as crop yield and biomass. These models are essentially working as a collection of many algorithms that requires several inputs specifying plant material, climate, and soil. Considering the current technological developments, one can assume that even a huge accomplishment such as this may just be the beginning. There are immense efforts towards increasing precision and automation in agriculture. In short term, we can expect the development of fully automated systems that govern every aspect of plant cultivation with upmost effectiveness. What really exciting is that for the long term there is no way of knowing the limits of this technology. Historical development of the growth analysis suggests that there is a possibility that someday modest achievements of the past concerning agronomy, statistics, and certain key technologies such as sensors, drones and energy conservation may collectively lead us to a bright future. In a bright future that our agricultural production may hopefully match increasing global food demand effortlessly, in a sustainable fashion that preserves our planet for future generations.

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CHAPTER 13

SESAME (*Sesamum indicum* L.) FARMING

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INTRODUCTION

Sesame (*Sesamum indicum* L.), a plant belonging to the Pedaliaceae family, is the oil plant with the highest production after sunflower in Turkey. In addition to being one of the first oil plants cultivated in history (Şahin, 2014; Öz, 2017; Batu & Batu, 2020), it has about 16 genera and about 60 species. However, not all of these plants have been listed taxonomically yet. Studies on this subject continue (Geçit et al., 2018).

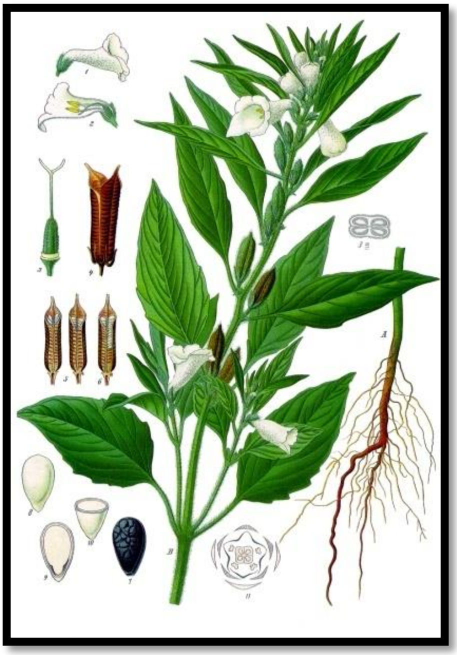

Figure 1: Sesame plant (https://en.wikipedia.org/wiki/Sesame)

The sesame plant, which has been cultivated since ancient times, was found in inscriptions thought to belong to the Babylonians around 2350 BC. According to the researches, it is said that sesame came to Anatolia from Mesopotamia and then spread to the Far East from here. It has even been concluded that this plant was brought to the Far East geography by the Turks (Geçit et al., 2018).

In Turkey, sesame plant is generally considered as the first or second crop in the Southwest Anatolia, Southwest, Marmara and Thrace regions. It has a plant structure suitable for alternation (Seçer, 2016; Yakar et al., 2021)

Table 1: Sesame production in Turkey over the years

Years	Sesame Sown Field (da)	Sesame Production (tone)	Sesame Yield (kg/da)
2016	289 332	19 521	67
2017	280 316	18 410	66
2018	259 858	17 437	67
2019	248 604	16 893	68
2020	256 663	18 648	73
2021	254 862	17 657	69

There are fluctuations in the production of sesame plants depending on the years in Turkey (Table 1) (TÜİK, 2022). However, this may be due to the effect of climatic and environmental factors. In 2019, 16 893 tons of production was carried out from the least area (248 604 decares) in the last six years, while 68 kg of product was obtained per decare. While a significant increase in sesame yield (73 kg/da) was determined in 2020, this value decreased in 2021 (69 kg/da).

In the world; According to the predictions of economists, the market share of the sesame plant, which is used by many sectors, will increase in the coming years. Sesame is produced mostly in North America, Europe, Asia Pacific, Middle East, Africa and Latin America (Figure 2). In addition to its oil, the sesame plant is also evaluated according to the color of its seeds (white, brown and black) and characteristics (with or without shell).

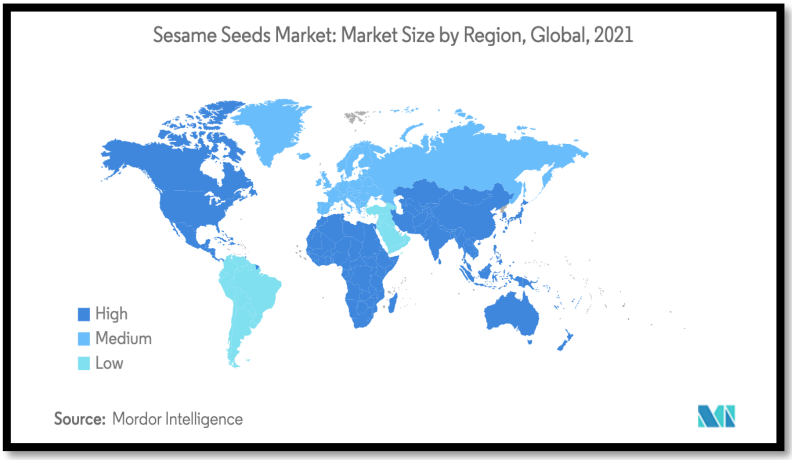

Figure 2: Market spread of sesame seeds in the world (https://www.mordorintelligence.com/industry-reports/sesame-seeds-market)

Cultivation of Sesame Plant

Sesame can also be grown in tropical and subtropical climates. However, since it is a heat-loving plant, it is adversely affected by sudden temperature changes (Yakar et al., 2021). The yield of the product is high in regions with an annual precipitation of about 500-600 mm. Apart from heavy textured, clayey or highly permeable sandy soils, sesame plants can easily grow in soils with medium texture (Geçit el al., 2018).

Sesame is grown in 90-120 days depending on the ecological and environmental conditions in which it is grown (Vurarak, 2021). It is an annual herbaceous oil plant with a tap root structure that can go down to a depth of approximately 1-1.5 m (Frankel & Hawkes, 1975; Bakal & Arıoğlu, 2013).

Figure 3. Sesame (*Sesamum indicum*) (https://www.britannica.com/plant/sesame-plant)

The resistance of this plant to dry periods is quite good, as it takes root very deeply and has a form that wraps around like a net. Depending on the climatic characteristics and conditions in which it is grown, the plant height can vary between about 30-180 cm. Since the sesame plant branches too much, it causes losses in the harvest of the product, which is not a desired situation by the producers. The leaf structure can vary on the same plant, or it can be hairy or hairless. The sesame plant, which has white, pink, purple flowers, blooms in a spiral from bottom to top. Sesame fruit, called a capsule, is in the form of a rectangular prism, narrowing from the bottom and top. Although the seeds are preserved in the capsules in general, seed losses are experienced due to excessive drying, especially during the harvest period. Sesame seeds, which can be white, yellow, yellowish, brown and black, have a weight of about 2.5-5 g (Ashri, 1989; Weiss, 2000; Geçit et al., 2018) (Figure 3). According to the relationship between the shell of the sesame seed and the substances it contains, the dark colored seeds contain higher protein and lower oil than the light colored ones (Baydar et al., 1999; Kurt, 2018).

Figure 4. Sesame seeds (https://www.herbazest.com/herbs/sesame

Sesame seeds are generally planted in March-April, although it varies regionally in a well-prepared and flattened soil (Geçit et al., 2018). In general, these seeds, which are placed in the seed bed with the spread sowing method. Unfortunately, decrease in yield when the smoothness in the living area is not fully ensured. For this reason, the use of mechanization opportunities suitable for the cultivation of sesame seeds will allow the producers to use less seeds and to obtain high yields. In addition to these, breeding studies are also carried out for the cultivation of varieties suitable for machine planting and harvesting (Figure 4).

The most important product record in sesame seeds is experienced at harvest (Vurarak, 2021). Seeds are poured from the capsules, which are particularly over-drying. Generally, the sesame plant, which is harvested by hand and sickle, is brought in heaps and the seeds in the capsules are poured. In machine harvesting, the high product losses prevent this valuable product from being used in more areas (Baydar, 2001; Öz, 2017). The sowing process is carried out with a row sowing machine with 40 cm between rows and 20 cm above rows (Geçit et al., 2018). The extracted seeds are then sieved, cleaned, placed in sacks, and the storage stage is started.

Evaluation of Sesame Plant

Although the amount of oil in sesame varies depending on the conditions in which it is grown, it generally contains 40-60% oil and 20-25% protein (Tan, 2011; Hatipoğlu et al., 2017) and also contains minerals such as Ca, Fe, Mn, Zn, Mg, Cu and Se (Demir, 2019).

Figure 5. Sesame oil benefits (https://www.lybrate.com/topic/benefits-of-sesame-oil-and-its-side-effects

Sesame oil has a high resistance to oxidation, especially because it is rich in oleic and linoleic acids at a rate of 40% each one, and antioxidant substances such as sesamol and sesaminol and tocopherols. In addition, the excess of unsaturated fatty acids (mono and poly) increases the quality of the oil. It also reduces the risk of cholesterol, hypertension and cardiovascular diseases in the blood (Yoshida & Takagi,1997; Arslan et al., 2014; Kurt et al., 2016; Hashempour et al., 2017; Bakal & Arıoğlu, 2020; Yakar et al., 2021). The amount and quality of oil obtained from sesame is directly related to environmental and cultural processes.

Sesame, which is a short day plant, changes the amount of oil in its body depending on the duration of light. While the length of the plant is short when the lighting time is reduced, the green part develops more as it benefits from more sunlight during the long day, and the number of capsules it forms increases (Öztürk & Şaman, 2012; Arıoğlu, 2014; Bakal & Arıoğlu, 2020).

Sesame has been used as a food product (margarine, bakery products, tahini, etc.) since ancient times, as well as in many different areas such as the health sector (for example: in the improvement of some intestinal disorders; balancing blood sugar, etc.), in the food of pets, and in the cosmetics sector (Baran & Gökdoğan, 2017; İbrahim, 2011; Kurt et al., 2016). Because, sesame oil has lots of benefits. Such as; helps in detoxification of skin, to fight stress and depression etc. (Figure 5). In addition, sesame pulp is also used in animal nutrition (Seçer, 2016; Erbil, et al, 2021).

As a result, it is necessary to increase the researches on breeding and mechanization required for the production of the sesame plant, which has come to the fore in terms of production and consumption in the world as well as in Turkey in recent years. In order to minimize the losses experienced during the harvest period, more research is needed on the development of new varieties or increasing the resistance of existing varieties according to regional and climatic conditions. In addition, the development of special planting and harvesting machines suitable for the structure of the sesame plant, and the testing of different production techniques will allow to increase the production volume. In this way, product losses experienced by producers will be prevented and more yields and thus income increases will be achieved.

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