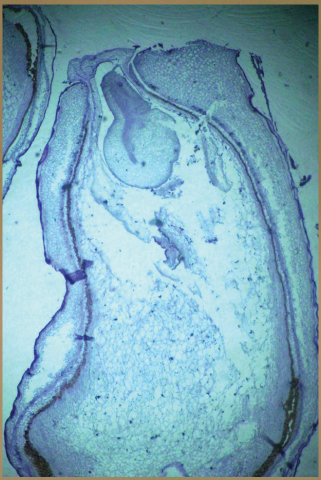

CURRENT AGRICULTURAL STUDIES in TÜRKİYE RESEARCH and REVIEWS

Editors
Assist. Prof. Dr. Sevil CANTÜRK
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RESEARCH and REVIEWS

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PREFACE

Agricultural studies cover a wide area from plant and animal production to conservation and using natural resources like soil, water, and the environment. Agricultural research areas face new challenges that shape the agricultural systems each year and the impact of global climate change. It has great importance to follow recent trends and technologies to increase the yield and quality of agricultural products, obtain healthy products, and manage natural resources. This book has been designed to evaluate the present situation in agricultural and food technologies, current developments, and new approaches.

The presented book is prepared with the contributions of researchers from some universities in Türkiye and consists of ten chapters in agricultural and food sciences. We hope that the book, including reviews and research, will be useful to researchers conducting scientific studies in related areas and the scientific world. We thank the valuable authors who contributed with their professional experiences in individual chapters. Finally, we would like to thank publisher İKSAD for their interest during the book project and publication process.

Editors

CHAPTER 1

GREEN FERTILIZATION WITHIN SUSTAINABLE AGRICULTURE: HISTORY, TYPES, AND MATTERS TO BE CONSIDERED

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1. INTRODUCTION

About 12 000 years ago, the transition from a hunter-gatherer to a settled lifestyle took place with land use for agricultural purposes. In the semi-nomadic lifestyle, the people who cultivated the lands near the areas where they stayed for a temporary period had to switch to a fully settled life due to the need for the use of larger lands for agricultural purposes and the storage of the obtained products (History 2018, Özdöl 2011; Grinin, 2007);. Since they were nomadic and their population was low, nature quickly repaired the footprints in the places they passed. With the transition to settled life, settlements like villages, towns, or cities emerged, and in their quest to meet needs, footprints left by the inhabitants were sometimes too big to be repaired. Even at the beginning of the Neolithic age, there is evidence that many villages or town residents faced famine, poverty, and disease due to abuse of agricultural land and mines and had to leave their settlements. (NTV 2009 a, b; Stories from the Stone Age 2004).

Although humankind could not realize it at the beginning of the neolithic age, he understood that intensely used agricultural areas were worn out over time. In other words, if he did not put back the nutrients exploited, he could no longer get yield from the same land.

To make the soil productive, the primary practices farmers have applied throughout history are as follows (Güneş, 2010; Karaman, 2012):

- Plowing, hoeing for aeration of the soil,
- Using human and animal excrement as fertilizer,
- Supporting the soil with applications such as wood ash, bone dust, sand, and sulfur to repair and strengthen both its productivity and its structure,
- Crop rotation practices to rest the soil and increase its productivity.

One of the applications for efficiency throughout history is Green Fertilization (Varanasi, 2021). This practice has been used since prehistoric times but came to the fore after the 18th century. The main reasons for this latency are the environmental and health problems that have increased with the transition to conventional agriculture. In this context, natural solutions such as green manure should be recognized and evaluated within the scope of sustainable agriculture.

2. HISTORY OF GREEN MANURE

Bringing soil fertility with green manure is a very old farm practice. Its value was understood by the Greeks and Romans, especially the Chinese, long before Christ. Green manure was not used among the peoples of northern Europe since the limited agricultural systems were insisted on by the kingdoms. For this reason, the practice was generally adopted by countries with a coast to the Mediterranean. This practice began to spread in Germany and England at the end of the 17th and the beginning of the 18th century, respectively. It was brought to the American colonies by British citizens. In the 20th century, the mysterious reasons for the nutritional and healing power of legumes for the soil were revealed (Pieters, 1927).

China: The earliest record of using grass or weeds as fertilizer is found in Han dynasty artifacts in China. It was during the Chou dynasty (1134-247 BC) that the value of soil improvement using green manure was realized. Tsi (500 BC) mentions that green manures were planted in the fifth or sixth month and turned over in the seventh or eighth month. He also says that the fertilization value of this application was as good as silkworm excrement and well-rotted farm manure. Wang Chen mentions that green manuring was common in the provinces of Kiangsu, Anhui, and the northern regions, in the Book of

Agriculture published in 1313. He also coined new terms by classifying cultivated plants used as fertilizers. Even today, many farmers in Kiangsu feed their rice fields with green manure techniques that are more than 300 years old (Pieters, 1927).

Greek and Rome: There is detailed information about green manure in Greek and Roman texts. Theophrastus (371 - 287 BC) (Shields, 2012) says that in some parts of Greece when the beans (*Vicia faba*) bloomed, they were turned under and buried in the ground. Roman writers on agriculture recommend planting lupine and beans on thin lands in many of their works. Varro (116-27 BC) mentions that some plants are grown not for use but because cutting and leaving them in the soil improves the soil structure; moreover, if the soil is too thin, it is necessary to harvest and bury the lupine and beans that have not yet matured (Pieters, 1927). Pliny the Elder (23-79) states that: "It is universally acknowledged by all writers that there is nothing more beneficial than to turn up a crop of lupines before they have podded, either with the plow or the fork or else to cut them and bury them in heaps at the roots of trees and vines." (Varanasi, 2021).

France: Agricultural literature in the Middle Ages is negligible, but there is evidence that the application of lupine for fertilizer continued uninterruptedly among Italian and southern French peasants from Roman times to the present day. According to Adam Dickson, Piero de Crescenzi (1724) reports that expert farmers in Tuscany sow lupines in July, August, and October: "They cut them down with spades and lay them in the furrows. There they saw the grain and covered the seed with the plow. The fields cultivated in this manner produce a plentiful crop next summer. However, the Milanese sow radishes thickly, and, when they have grown up, turn them underground; others sow lentils and turn them underground when they have arrived at their full growth." (Pieters 1927).

Germany: Green manuring was not included in the "three-field system" of the Middle Ages in Northern Europe. At the end of the 17th century, green manure began to appear in Germany, and lupines and horse beans started to be used for green manure. It was customary to plant peas in distant fields and plow them under when they began to bloom to make the soil fluffy and soft. The spread of the lupine culture in sandy soils was also reported in 1817. Since Albert Schultz (Schultz-Lupitz) introduced this practice so effectively, Nolte calls him the "father of modern green manure in Germany" and ranks his work after the works of Liebig. In the middle of the nineteenth century, interest in green manure reached such a level in Germany that agriculture without cattle and using green manure to replace stable manure were encouraged (Pieters, 1927).

England: Green manuring came to the fore in the early 18th century when Mortimer mentioned the use of buckwheat as green manure for wheat in England. Adam Dickson mentions that in England, buckwheat, peas, clover, and other legumes were sometimes planted to be plowed for fertilizing (Pieters, 1927).

America: The application of green manure to the American colonies came from England but was not adopted very quickly. Jared Eliott acknowledges the value of red clover in rehabilitating weathered soil. "Another way of mending land is what they call in England green dressing; this is by sowing buckwheat, oats, or rye, and when it is grown up and is full of sap, they plow it in, after this let it be till fully rotten, then plow again and sow your wheat.". At the end of the eighteenth century, green manure was applied to some extent in Maryland and Virginia. However, interest in green manure increased in the early 19th century and was adopted in some formerly inhabited areas of the United States within a few years. In 1856 Reinbold published an exciting study advocating harvesting and seeding all kinds of plants, many of which are now considered among the most troublesome weeds, for an intense and rich green manure; after that, different authors produced works on the same subject (Pieters, 1927).

Although the use of legumes as green manure goes back to prehistoric times, the dynamics underlying its power to improve the soil were discovered in the 20th century. In his book "Soil Fertility and Permanent Agriculture" in 1910, Cyril Hopkins states that "Legumes can fix their own Nitrogen if they have the appropriate bacteria."; thus, he uncovers a mystery (Varanasi, 2021).

3. WHY NOW?

Although it is an old practice, it was only after the 18th century that the name of green manure was widely heard and its importance understood. The main reason for this is the transition to conventional agriculture to meet the basic food needs of the masses due to the gradual increase in the population after the Industrial Revolution. Consequently, the adverse outcomes of using chemical fertilizers and pesticides, which were seen as blessings once, were encountered. The first reason for the excessive use of these supplements is the lack of knowledge or misleading of the farmers. Individuals who increase their crops using chemical fertilizers and pesticides tend to think that the more supplements, the more product to get. The increasing need for water, agricultural fertilizers, and pesticides of genetically modified seeds is another reason for the excessive use of these supplements (Hurt, 2020).

Unconscious consumption of fertilizers and pesticides, which are used to restore soil fertility and prevent pests, has ended in the accumulation of chemical substances in the soil, underground water resources, and the bodies of living beings. Therefore, ecological and health-related deteriorations were inevitable (Sönmez et al., 2008). Most nitrogen fertilizers are not absorbed products and penetrate ground and surface waters. 50% of the nitrogen fertilizer applied to the soil is used by the plants, 2-20% is lost by evaporation, 15-25% reacts with the organic compounds in the clay soil, and the remaining 2-10% mixes with the surface and ground waters. Today, the problem of nitrates accumulating in groundwater is a problem in a global context. In 22% of the cultivated areas in Europe, the nitrate concentration in the groundwater is above the recommended value of 11.3 mg/L. This figure is 23 mg/L in European countries and 45 mg/L in the United States (Yılmaz et al., 2017; Savcı, 2012).

Due to the buffering power of the soil, the damages caused by unconscious fertilization and spraying are noticed in the long term, and this situation poses an even greater danger. The longer the soil structure has degenerated, the longer it will take to repair. One of the most painful examples is the increase in the acidity level of agricultural areas on the Black Sea coastline, where tea is cultivated intensely due to one-way ammonium sulfate fertilization in Türkiye. Studies showed that soil reaction (pH) in tea gardens varies between 3.29 and 5.59, and the pH is below the critical limit of 4.5 at 53.95% (Taban et al., 2015). Because of the nitrogen fertilizers applied for more than 25 years in Nevşehir, the acidity in the potato fields increased 100 times, and the pH decreased to 2 (Savcı, 2012).

One of the adverse effects of chemical fertilizers on the environment is salinization. The predominance of Na ions in the physical structure of the soil causes a decrease in the yield and quality of the plants. In Antalya / Demre, 93% of tomato-grown greenhouse soils at 0-20 cm and approximately 90% at 20-40 cm are included in the medium and high saline (Sönmez and Kaplan, 2004). Similarly, it was determined that the salt content of greenhouse soils where cucumbers and tomatoes were grown in Antalya / Kumluca and Finike regions increased during the growing period. Studies show that fertilizer applications are one of the basic reasons for the increase in salt content (Akay and Kaplan, 1995).

Another major problem is that fertilizers and pesticides used in agriculture leave significant amounts of toxic elements in the soil and the plant. These toxic elements are mainly heavy metals such as cadmium (Cd), lead (Pb), nickel (Ni), arsenic (As), and copper (Cu). Reaching toxins into the soil is due mainly to phosphorus fertilizers and their raw materials. Studies show that the heavy metal contents of raw phosphate rock imported for phosphorus fertilizer production are significantly high; moreover, it has the highest concentration of Cd and As compared to other fertilizers. Cd, Pb, Ni, and As are also present in phosphoric acid, which has replaced raw rock phosphate in recent years. However, Pb in compound fertilizers; As in DAP and TSP; Cd in DAP, TSP, and Composite fertilizers is above the critical limit (Köleli and Kantar, 2006).

The intensive greenhouse cultivation in the Mediterranean Region causes the consumption of fertilizers and pesticides in high quantities. The use of Cu-containing preparations increases the Cu content of soils. Sönmez et al. (2006) applied Cu fertilizers in different concentrations and detected Cu accumulation in the greenhouse soils and plant leaves at the end of the growing period. Heavy metal accumulation in the soil and, thus, in the plant certainly negatively affects the development of the vegetative and generative organs of the plants (Singh and Kalamdhad, 2011). Also, people have been intensely exposed to heavy metals in the last 50 years due to various industrial products such as cosmetics, hair care products, dyes, Pb in tap water, mercury amalgams used for filling, and chemical residues in toothpaste. When heavy metals we take with food are added to all these, many serious health problems, from cancer to thyroid, from neurological disorders to infertility, are encountered (Özbolat and Tuli, 2016).

Eutrophication is the gradual increase in the concentration of phosphorus, nitrogen, and other plant nutrients in an aging aquatic ecosystem, such as a lake. The productivity or efficiency of such an ecosystem naturally increases as the amount of organic matter decomposes into its building blocks over time. This material enters the ecosystem primarily with the runoff from the soil, which carries the debris and products of the reproduction and death of terrestrial organisms; therefore, high populations of algae and microscopic organisms begin to develop. Researches show that there has been an increase in these abnormal and toxic plant species in the last 15 years. These species usually thrive on the surface and prevent light penetration and oxygen absorption, which are necessary for underwater life. Eutrophic waters are generally turbid and less likely to harbor large animals, such as fish and birds, than non-eutrophic waters due to unfavorable conditions (Britannica, 2022a). The primary sources of nutrients that cause accumulation are domestic, especially the ones coming from sewage, wastes, fish farming, and agricultural wastes caused by unconscious fertilizer use (Ansari and Gill, 2014).

Organic matter loss is another issue fueled by conventional agriculture. Soil fertility depends on the amount of organic matter. The part with the highest amount of organic matter and plant nutrients are kept in the top 0-25 cm of the earth; however, this small segment contains 95% of the nitrogen and 25-50%

of the phosphorus stored in the soil. On the other hand, organic matter lost due to erosion due to degenerated soil structure caused by intensive use of fertilizers and pesticides is 1.3-5 times more than the rest of the organic matter kept in the earth (TEMA, 2022).

The decrease in the amount of organic matter in the soil from 1.4% to 0.9% causes a 50% yield loss in grain production. Between 1960-1995 (Hurt, 2020; Jain, 2018; Pingali, 2012), when the green revolution took its momentum, the amount of fertilizer used in wheat production increased seven times. While one kilo of chemical fertilizer used in wheat production in the early 1960s increased the product by 700 kg per hectare, this amount decreased to 250 kg/ha in 1995. It is seen that the use of chemical fertilizers alone is not sufficient without the addition of organic matter; on the contrary, it causes rapid mineralization of valuable organic matter stored in the soil. While the soil should fix the carbon (C), the opposite emission occurs, and the atmosphere/earth C balance is disturbed. In other words, deterioration of soil structure causes loss of organic matter, and this causes greenhouse gas emissions. Today, with the correct agricultural practices, it is predicted that the European Union (EU) lands can store 73-79 billion tons of C, which is approximately 50 times the annual greenhouse gas emissions of the EU. However, due to intensive tillage, European cultivated lands released an average of 0.45 tons of CO_2 per hectare into the atmosphere in 2009, most of which was due to land-use change and intensive tillage-agriculture (TEMA, 2022; FAO, 2015a; Detwiler, 1986). It is also known that organic matter affects the solubility of certain minerals (Berggren, 1995).

Intensive tillage is another factor that damages the soil structure and causes the organic matter to be lost. Today, 150 tons/ha of soil is lost annually due to erosion caused by improper and unconscious tillage. In order to prevent these losses, protective tillage methods are now being developed. The most natural method is cultivating the soil without intensive hoeing. The aim here is to reduce the number of processes and to keep the soil surface covered with vegetation as much as possible. In many countries, especially Southern Brazil, North America, New Zealand, and Australia, numerous movements have started to protect the soil, and studies on farming by imitating nature without cultivating have become widespread (FAO, 2022; Mollison, 1997).

There are numerous advantages of keeping the top of the soil wholly covered. The mulch layer protects the soil from the destructive effects of rain and wind, ensures the continuity of soil moisture and temperature, and creates a habitat for soil inhabitants and microorganisms. After a while, in a soil left to itself in nature, a natural mulch layer consisting of creeping plants and natural wastes forms between the trees and the dominant vegetation. Besides protecting the soil, the mulch layer feeds it by blending with it over time due to its decomposition and acts as a buffer for the organic material, water, and nutrients. Moreover, if the soil structure is strengthened with correct agricultural practices and supported by a polycultural perspective, the need for cultivation will be less. With strengthening soil fauna, increasing natural processing and ventilation of the soil due to the activities of macro and microorganisms causes this positive outcome. The effects of these organisms on the soil are called "biological processing.” Today, biological processing is interrupted due to intensive agricultural practices; it is not compatible with mechanical machining (FAO, 2022; Mollison, 1997).

In light of all these mentioned, it can be said that; the agricultural and health problems fueled by conventional agriculture required a closer look at the soil (Pingali, 2012). In this case, green manure applications, which have been used since prehistoric times, appear as an excellent solution to the numerous problems that come with chemical fertilizers and other soil cultivation practices.

4. WHAT IS GREEN MANURING, AND HOW DOES IT CONTRIBUTE TO THE SOIL?

"Green manuring is the mixing of some plants with the soil, either by growing them in place or after being grown in another area." (Gulec et al., 2018)

"It is called the bringing of green manure plants under the ground at a certain period of their development while the plants are still green." (Karakurt, 2009).

"Crop grown and plowed under for its beneficial effects on the soil and subsequent crops, though it may be grazed during its growth. These crops are usually annuals, either grasses or legumes. They add nitrogen to the soil, increase the general fertility level, reduce erosion, improve the physical condition of the soil, and reduce nutrient loss from leaching. They are usually planted in the fall and turned under in the spring before the summer crop is sown." (Britannica, 2022b)

"By green manuring is meant the turning under of a green crop, for the enrichment of the soil. Some German writers have limited this definition to the turning under of legume crops, but there seems no warrant for this restriction. Strictly speaking, the green crop must have been produced for the purpose of being turned under." (Pieters, 2006)

"Green fertilization is the burying green plant material (excluding product residues) grown in situ or brought from elsewhere to enrich the soil. The plants used for this work are called green manure plants." (Taban and Turan 2012)

"It is not possible to distinguish sharply green manuring from cover cropping, though each term has a basic meaning of its own. The term "cover crop" was first used by Prof. L. H. Bailey to designate a crop especially planted to cover the ground in winter and to serve as a protection to the roots of trees. When such a cover crop is rye, or clover, and is turned under in spring, it becomes a green-manure crop" (Pieters, 1927).

In addition to the stem and leaves of the plant, the root is added to the soil as organic matter. In other words, soil regains the nutrients absorbed by the plants as a result of their decomposition. In particular, leguminous green manure plants absorb the free nitrogen from the air, and nitrogen fixed by the legumes is mixed with the soil at the end of its decay. On the other hand, since this watery and organic material-rich plant will take a long time to decompose, its effect continues for several years, not just one year, depending on the climate and other conditions. The effect of green manure lasts longer in humid and cool climates than in hot and arid ones and is effective for a shorter time in sandy soils than in clay soils (Güleç et al., 2018). Many factors, such as soil pH, the C: N ratio of plant material, and the condition of other nutrients in the soil, are also effective in the mineralization of green manures (Taban and Turan, 2012).

The planting time naturally varies according to the selected plant and the green manure type. In the temperate climate zone, it is generally planted in autumn or early spring and overturned in May at the latest; it rots when there is enough moisture, and sowing can be done in the fall.

5. PLANTS USED IN GREEN FERTILIZATION

Some scientists mean only legumes when they say green manure plants, but this narrows the definition. If the aim is to restore the minerals and organic matter lost to the soil, any plant material such as grass, weeds, clover, cowpea, or crop residues fulfills this purpose. Moreover, this is valid for green plants and dry materials (Güleç et al., 2018; Pieters, 1927).

To be evaluated in general, green manure plants can be divided into two classes considering the type or season that they plant (Taban and Turan, 2012):

Winter

Winter legumes: Hairy vetch, crimson clover, spotted medick, and peas

Winter grain: Rye, wheat, grass, oats, and barley

Summer

Summer legumes: Alfalfa, honey clover, red clover, soybean, and cowpea

Summer cereals: Oats, barley, summer wheat, buckwheat, millets, maize, and elephant grass

6. WHAT ARE THE BENEFITS?

Green manure has numerous benefits on many different topics listed below.

Soil tilth: The value of the organic matter in providing nitrogen and improving the physical condition of the soil is indistinguishable; that is, the same organic matter necessarily fulfills both functions. Along with all kinds of organic matter such as non-legumes, plant residues, stubble, roots, and grass sod also contributes to the physical development of the soil on par with legumes. After green manuring studies in India, Lander et al. (1923) found that the main factor in increasing wheat yield was the improvement in the physical condition of the soil, and also non-legume plants were as effective as legumes for green maturation.

Green manure prepares the soil as a seedbed suitable for planting, especially since it improves its physical structure due to its organic matter. Loamy soil particles break down easily; there are no hard lumps in them; It ideally preserves water, air, and beneficial nutrients for the plant. Sandy soil is too porous and cannot hold water and micro/micro minerals. On the other hand, fine soil particles of clay tend to stick together, so they are airless and cannot absorb moisture. Clay soils with insufficient organic matter become hard as they dry, the seed bed remains lumpy, it becomes difficult to plant throughout the season, and water puddles form onto it during irrigation. In this case, the soil particles are tried to be loosened by hoeing or plowing; its structure deteriorates, and the situation worsens over time. Thus, it takes a long time for irrigation water to penetrate the soil. With the addition of organic matter, the disadvantages of both thin and thick soils are eliminated (Karaman, 2012; Güneş et al., 2010; Pieters, 1927).

The organic matter makes the soil suitable for cultivation with its humus and coarse organic matter. Humus is rich in colloids and therefore has excellent absorbent power. Fine humus particles tend to surround each mineral particle with a thin protective layer so it can absorb and retain moisture and bases. This absorption power is vital in regulating phosphorus and potassium sources in soils. Humus not only helps retain moisture in sandy soil but also binds sand particles together, reducing their tendency to fly and drift; In this way, it also prevents erosion. Besides the part of the organic matter represented by humus, another part consists of a certain amount of undecomposed or partially decomposed coarse organic matter. This part improves aeration in clay soils and delays water infiltration in coarse sands, which is extremely important in such soil types. Since there are no weeds and crop roots in the orchards where commercial production is carried out, organic matter is not added to the soil naturally. Over time, most of the original organic matter is destroyed by soil organisms, and the physical condition of the soil gradually deteriorates. When this happens, the soil structure cannot be improved even if commercial fertilizers are added (Karaman, 2012; Güneş et al., 2010; Pieters, 1927).

Most of the time, it is not possible to fully distinguish the role of legumes as cover crops from the role of green manure. While serving as a cover to protect the soil from being washed away, it eventually mixes with it as green manure; hence it adds organic matter and improves the texture and water-holding capacity of the soil. In general, deep-rooted legumes tend to make the soil more porous with their roots; therefore, canals are provided for the roots of the main crop to grow. This physical soil improvement for the main crop is perhaps as significant as the benefit from the additional nitrogen provided. In addition, green manure is effective in improving soil structures that are compressed and degenerate due to reasons such as transportation, intensive use, and heavy grazing (Pieters, 1927).

Plowing draft: While the difference in traction in sand or light sandy loam is not very noticeable, the draft can increase significantly in heavy clays as organic matter is lost and the soil hardens. Studies show that the soil with the highest organic matter has the lowest draft and that with the lowest has the highest. It may not make much difference in loamy soils, but definite improvement in the draft is detected in heavy soils after a few green manure crop cycles (Pieters, 1927).

Organic matter decomposition on soil mineral solubility: In fact, most soils have enough potential plant nutrients to produce nearly unlimited yields, but in most cases, these minerals become available over a very long time. The reason is that plant nutrients originate from mineral particles of soils, mainly weathered rocks, and it takes a long time for them to become beneficial for plants and living things. Only the solvent liquids secreted by the plant roots and the increased microorganism activities accelerate this process. If the soil lacks to maintain beneficial elements, choosing green manure instead of commercial fertilizers is suitable as it increases microorganism activities. Soil microorganisms affect beneficial plant nutrients, convert organic nitrogen to nitrates, and fix atmospheric nitrogen. The decomposition of plant material leads to carbon dioxide, which has a pronounced solvent effect on soil minerals. Studies show that carbon dioxide produced in decay increases the solubility of phosphates and the usefulness of potassium. Legume roots especially significantly affect the solubility of beneficial nutrients. Therefore, it can be said that one of the indirect benefits of green manuring is the increased availability of soil minerals (Gondal et al., 2021; Pieters, 1927).

Shade effect: Some researchers even argue that the physical effect of shading is the most significant effect produced by green manure plants. When the soil is covered with plants rather than bare, it is less affected by many natural events (Aguiar, 2019). As a result of green manuring, the movement of microorganisms is facilitated, and the soil becomes suitable for agriculture, as it remains constantly covered, shaded, sheltered, and brittle. A growing crop may dry out the soil, but such water loss has a different effect than the effect of water loss by evaporation from the surface of terracotta soil. In this sense, due to green manuring, the soil surface is not cooked and hardened by intense sun rays and does not become compressed due to heavy rains. In arid regions, the shade effect of the summer cover plant in orchards is more critical because, without such shade, the sun reflected from the terracotta can cause burns not only on the soil but also on young trees (Pieters, 1927).

Soil pH: Growing crops, especially legumes, tend to lower the alkaline content of the soil by shading it; that is, it causes reduced evaporation from the surface. It should not be forgotten that organic matter in acidic soil will increase the acidity even more while decreasing the alkalinity in alkaline soil (Karakurt, 2009; Pieters, 1927).

Weeds: When green manure acts as a cover crop, its effect on keeping weeds is a little added benefit, among other effects, and this function will only be fully fulfilled when the green manure crop is in lush growth and thus completely covers the soil (Karakurt, 2009; Pieters, 1927).

Root activity: The effect of the root activity of green manure plants, especially legumes, is essential for a healthy soil structure (Burridge et al., 2020; Vandez et al., 2008). Schultz (1987) found that lupines can penetrate 80 or more centimeters, puncturing a hard pan layer about 40 cm deep. Shultz grew lupine as a stubble crop in a part of the experimental field, while in the rest, he used stable manure and chemical fertilizers to provide as much nitrogen as lupines. He cultivated potatoes in both areas and determined that the potatoes grown in the land fertilized with green manure were dark green and healthy and obtained 9437 kg per acre. Moreover, it was observed that where the lupines grow, the potato roots follow the channels left by the lupine roots, thus absorbing more water and minerals and can progress to the subsoil layer. On the other hand, 5915 kg of lower tubers were obtained from the land fertilized with stable manure and chemical fertilizers, and the roots of the potatoes could not penetrate the hard soil layers; therefore, they suffered from thirst and were of low quality (Pieters, 1927).

Depth of roots: It is impossible to generalize that legumes take root deeper than other plants used as green manure. Researchers have found that grains go as deep as legumes when it comes to the depth of root systems. However, red clover has a greater root mass in deeper soil layers than in grains. While some maize roots go down to a depth of 120-180 cm, most of their root mass remains close to the surface. Clover contains most of its root system at a depth of under 60 cm, penetrating up to 120-150 cm in its first year. Most pea and vetch roots are close to the surface, while soybean and cowpea roots may extend deeper. Sweet clover has a particularly strong taproot, which is known to assist financially in improving the drainage of hard soils. Small stone clover (*Melilotus indica*) penetrates 244 cm deep in citrus groves in Southern California. There is evidence that the roots of legumes such as clover, sweet clover, red clover, soybean, cowpea, and lupine penetrate the soil, opening it up, especially for plants with shallow roots, such as maize and potatoes. It should be added that most legumes leave a more considerable root residue in the soil than grains (Pieters, 1927).

Decomposition products: When new organic matter is buried in soil, microorganisms break complex proteins and carbohydrates down into their building blocks. Carbohydrates are mainly used for energy purposes. Some proteins can be used as a nitrate source by rapidly growing plants to meet their nitrogen needs. While some of these protein degradation products are harmful, they are rapidly oxidized in healthy soil. Therefore, it is vital to protect the physical structure of the soil with methods such as liming and green manuring (Karaman, 2012; Güneş et al., 2010; Pieters, 1927).

Farm inputs: Where animal manures are insufficient, green manuring can be considered an organic matter source (Güleç et al., 2018); therefore, chemical fertilizer inputs can be reduced in this respect.

7. TYPES OF GREEN MANURES

Green manures can be classified under four main types as follows:

Main crop: If green manure plants are grown as the main plant for fertilization, the field is covered with them throughout the year, and no other crops can be cultivated. Such a practice is inherently expensive because the farmer pays for the seed, spends his labor on the crop, and does not derive a direct and immediate income from the land he occupies. For this reason, this application can be applied in humus-poor light sandy soil, which is economically unsuitable. If it is to be grown as the main crop, it is possible to grow two crops in that field in the same year. For example, rye is planted in autumn and brought under the ground in spring or early summer; afterward, broad beans or millet are planted, and these plants can be brought under the ground in autumn. This application is especially recommended in fallow areas (Taban and Turan, 2012; Karakurt, 2009).

Companion crop: Companion crops are preferred to help or accompany the main crop and remain in the soil even more. Companion plant is planted with grain, the main crop; after the grain is harvested, it continues to develop and turns under the ground in autumn. For example, it is common practice to plant alfalfa together with winter cereals or spring cereals. After the grain is harvested, the clover covers the ground, and if the season is good, it can even grow enough to cut a ton of straw per acre. Such a crop is secondary to the grain or companion crop and costs the farmer nothing but seed and little labor of planting. This is perhaps the most economical form of green manure application and can be very effective. Companion plants can also be used in orchards. Both tree and green manure plants occupy the soil together, so the soil surface is not left empty; hence it is protected and nourished. If the soil is fine, companion plants should be planted as early as possible in early March so that the plant uses the water accumulated in the winter and develops quickly. However, sowing can be done in the middle or end of April if the soil is in its ideal condition and hold enough water. If it is to be grown with winter grains, sowing should be done with a seeder after the soil is fluffed with a hoe and rake (Taban and Turan, 2012; Karakurt, 2009; Pieters, 1927).

Catch crops/stubble crops: In this system, green manure plants are planted as catch crops on the stubble after harvesting the main crop in summer. In the Central Anatolian Region, the seeds used for green manuring are planted by spreading (by hand or with a fertilizer spreader) before the fallow and then are buried with a plow. These plants are turned under the ground in autumn or next spring. The cost and workload of stubble crops are more expensive than companion crops since it requires a second plow and sowing process. However, it is preferred in combating weeds. The disadvantage of this system is that the catch crop plants consume considerable water. There should be no water shortage for these plants to grow on stubble. Regions that receive large amounts of early summer and autumn rains or have irrigation facilities are suitable for this fertilization (Taban and Turan, 2012; Karakurt, 2009; Pieters, 1927).

Another point to be considered is that the green manure plant must be a fast-growing or annual plant. These crops, which have a short growth period and are grown after the main crop is harvested or between two main crops, such as potatoes and wheat, are also called intermediate crops or stolen crops. Mustard, rape, turnip, cowpea, soybean, and buckwheat are among the common stubble crops (Pieters, 1927).

Winter cover crop: Unlike other green manure applications, this system does not depend much on soil and climate conditions. It is applied in October/September and is used in April/May. The point to be considered is the suitability of the selected plants. If the plant leaves the field late, it forms a large amount of biomass but uses the water of the following plant. The plants, which leave the field quickly, do not exploit much water, but their biomass may not be sufficient as a fertilizer since they are underdeveloped. Green manure plants are grown as winter cover crops in Türkiye, especially in coastal areas (Taban and Turan, 2012; Karakurt, 2009; Pieters, 1927).

8. THE NUMBER OF SEEDS TO BE PLANTED PER DECARE

The number of seeds to be planted using seeders per hectare should be as follows: 140-160 kg for vetch, 200-250 kg for broad bean, 180-200 kg for grains, 50-80 kg for mustard, 80-100 kg for sunflower, 100-120 kg for fodder peas, 120-140 kg for cowpea and 100-120 kg for soybean. If spread seeding is applied, the number of seeds should be used 20% more than with seeder (Karakurt, 2009).

9. MATTERS TO BE CONSIDERED

Many factors affect the success of the green manure plant and need to be considered during selection (Taban and Turan, 2012; Karakurt, 2009).

The type of green manure should be meticulously chosen considering the intended use.

Resistance to stress factors such as thirst, heat, and cold should be considered.

The development period should be evaluated considering the intended use. If it is the main crop, it stays throughout the season; however, in some other green manure applications, it is crucial that the development is rapid and does not occupy the soil for a long time.

The biomass of green manure plants is expected to be as much as possible.

The root depth and width of the selected plant are expected to be as long and wide as possible.

The organic matter amount and nitrogen fixation ability of the selected plant are among the primary considerations. Legumes are mostly preferred as green manure plants due to their nitrogen fixation ability and high amount of organic matter.

Water demand is also essential since the amount of water consumed by legumes to form dry matter is relatively high compared to grains. Therefore, water supply is vital in the region where legumes are preferred.

Compatibility with the region and climate is essential for protecting farmers economically and preventing unnecessary labor waste.

Compatibility with companion crops should be finically evaluated. Although leguminous plants are preferred, it would be more appropriate to grow legumes and non-legumes together to obtain more organic matter. Mixing deep and shallow-rooted plants, grains, and legumes should be preferred in this case. Oats and peas, rye, and vetch are good examples of companion planting. These plants can develop together quickly, form a large amount of biomass and show better development in low-fertile soils.

A suitable cultivation method should be used for the healthy development of the selected plant.

The correct time for turning the green manure plant under the ground is at the beginning of flowering. If this process is delayed, the expected benefit from green manure will decrease.

Burying method must be applied carefully. If the plant is not wholly turned under the ground or cut and left onto the soil, some of the nitrogen in its structure fly away. Therefore, the harvested plant must be turned under the ground with the help of a disc harrow or plow.

The organs of the plant, such as roots, stems, leaves, and flowers turned under, are also essential factors to be considered since they affect the quality of green manure.

Physical, chemical, and biological features of the soil must be considered.

Value as a fodder plant must be evaluated regarding nutrition and economic benefits.

Value as a seed must be evaluated in terms of quality and economy.
Ease of being turned under and the decomposition process are also important.

10. CONCLUSION

Soil is currently considered in the category of "non-renewable energy source." Today, 33% of soils are degraded at moderate and high levels due to soil erosion, salinization, compaction, acidification, and chemical pollution. The current rate of soil degradation threatens the capacity of future generations to meet their most basic needs (FAO, 2015b). This brings us to the following point; More than ever, we need organic and sustainable solutions to restore the soil productivity lost due to agricultural activities. This is why green manure has been used for centuries but has gained popularity recently.

Green manuring contributes significantly to improving soil structure and restoring lost organic matter and minerals to the soil. Other benefits include increasing the yield and quality in the orchards and pastures and providing economic comfort for the farmer by reducing chemical fertilizer, pesticides, and labor inputs. The selection of plants suitable for the region and the intended target and correct learning and application methods are essential. Therefore, local research and applications need to be developed to determine the appropriate companion plants and methodology related to the subject.

REFERENCES

Aguiar, A., Robinson, S., French, K. (2019). Friends with benefits: The effects of vegetative shading on plant survival in a green roof environment. *PLoS ONE* 14(11): e0225078.

Akay, S., Kaplan, M. (1995). Soil salinity and seasonal variation of greenhouses in Kumluca and Finike regions. *İlhan Akalan Soil and Environment Symposium*. 289-298

Ansari, A.A., Gill, S.S (2014). Eutrophication: Causes, Consequences, And Control. Ed: Ansari AA, GillSS. Springer. 2(273). ISBN: 978-94-007-7813-9

Berggren, D., Mulder, J. (1995). The role of organic matter in controlling aluminum solubility in acidic mineral soil horizons. *Geochimica et Cosmochimica Acta* 59(20): 4167-4180.

Britannica (2022a). Eutrophication, https://www.britannica.com/science/eutrophication Date of access: 8.8.2022

Britannica (2022b). Green Manure, https://www.britannica.com/topic/green-manure Date of access: 8.8.2022

Burridge, J.D., Rangarajan, H., Lynch, J.P. (2020). Comparative phenomics of annual grain legume root architecture. *CropScience* 60: 2574–2593.

Detwiler, R.P. (1986). Land use change and the global carbon cycle: the role of tropical soils. *Biogeochemistry* 2(69).

FAO (2022). Conservation Agriculture, Fact Sheet. https://www.fao.org/3/cb8350en/cb8350en.pdf Date of access: 08.08.2022.

FAO (2015a). Status of the world's soil resources, Rome, Italy. 650, ISBN: 978-92-5-109004-6.

FAO (2015b) Soil is a Non-renewable Resource, Fact Sheet. Date of access: 08.08.2022 https://www.fao.org/documents/card/es/c/ec28fc04-3d38-4e35-8d9b-e4427e20a4f7/

Grinin, L.E. (2007). Production revolutions and periodization of history: A comparative and theoretic-mathematical Approach. *Social Evolution and History* 6 (2): 75–120.

Gondal, A.H., Hussain, I., Ijaz, A.B., Zafar, A., Ch, B.I., Zafar, H., Sohail, B.D., Niazi, H., Touseef, M., Khan, A.A., Tariq, M., Yousuf, H., Usama, M. (2021). Influence of soil pH and microbes on mineral solubility and plant nutrition: a review. *International Journal of Agriculture and Biological Sciences*. 71–81.

Güleç, H., Pilanalı, N., Kalınbacak, K., Keçeci, M., Özcan, H. (2018). *TR Ministry of Agriculture and Forestry Fertilization Guide*. Nevşehir. 62.

Güneş, A., Alpaslan, M., İnal, A. (2010). Plant Nutrition and Fertilization. A.Ü. Ziraat Fakültesi, 576, Ankara.

History.com editors. 2018. The neolithic revolution. https://www.history.com /topics/pre-history/neolithic-revolution Date of access: 08.08.2022.

Hurt, D. (2020). Green revolution in the global south, science, politics, and unintended consequences. Ebook, 288.

Jain, H.K. (2018). The Green Revolution: History, Impact, and Future, SP LLC, USA.

Karakurt, E. (2009). Green fertilizers and fertilization in terms of soil fertility. *Journal of Field Crops Central Research Institute, Compilation (Review)*18 (1-2): 48–54.

Karaman, M.R. 2012. Plant Nutrition, Gübretaş Guidebook series 2. Ankara.1066

Köleli, N., Kantar, Ç. (2006). Heavy metal hazard in phosphorus fertilizers. *Journal of Ecology*, 9: 1-5.

Lander, P.E., Wildson, B.H., Mukand, M.A. (1923). A study of the factors operative in the value of green manure. *Agr. Research Inst. Pusa Bul.* 149.

Mollison, B. (1997). Permaculture: A Designer's Manual, Tagari, 576.

NTV World History. (2009a). Chapter: Prehistory Edt. Derya Tulga, Berlin, 544.

NTV Knowledge Cube. (2009b). Chapter: Cultural History of the World. Eds. Mustafa Alp Dağıstanlı, Berlin, 512.

Özbolat, G., Tuli, A. (2016). Effects of Heavy Metal Toxicity on Human Health. *Archive Source Review-Journal (AKTD)* 25(4): 502 – 521.

Özdöl, S. (2011). Religion and Social Structure in Southeast Anatolia in the Pre-Pottery Neolithic Age. *Journal of Historical Studies* 26(1): 173-199.

Parthenon Films. (2004). Stories from the Stone Age. https://www.youtube.com/ watch?v=-7bqi70B3tE. Date of access: 08.08.2022.

Pieters, A.J. (2006). Green manuring: Principles and practice, Braunworth and Co. Inc. NY. 267

Pingali, P.L. (2012). Green Revolution: Impacts, limits, and the path ahead. *PNAS* 109 (31): 12302-12308

Savci, S. (2012). Investigation of the effect of chemical fertilizers on the environment. *APCBEE Procedia* 1: 287-292.

Schultz, L. (1987). Zwischenfruchtbau auf leichtem Boden. *Arb. Deut. Landw. Gesell.* 7. 3d Auflage.

Shields C. (2012). The Oxford Handbook of Aristotle. Oxford: Oxford University Press. ISBN 978-0190244842.

Singh, J., Kalamdhad, A.S. (2011). Effects of heavy metals on soil, plants, human health, and aquatic life. *International Journal of Research in Chemistry and Environment. Review Paper.* 1(2): 15- 21.

Sönmez, İ., Kaplan, M., Sönmez, S. (2008). Effects of chemical fertilizers on environmental pollution and solution suggestions. West Mediterranean Agricultural Research Institute *Derim Journal*. 25(2): 24-34.

Sönmez, S., Kaplan, M., Sönmez, N.K., Kaya, H., Uz, İ. (2006). High levels of copper application to soil and leaves reduce the growth and yield of tomato plants. *Scientia Agricola* 63(3): 213-218.

Sönmez İ., Kaplan M. (2004). Determination of salinity of soil and ırrigation waters of greenhouses in Demre region. *Journal of Akdeniz University Agriculture Faculty* 17(2): 155-160.

Taban, S., Turan, M.A., Soba, M.R., Taşkın, M.B., Balcı, M., Kabaoğlu, A., Özer, S.P., Kalcıoğlu, Z., Müezzinoğlu, N. (2015). Determination of the boron concentration of the tea cultivation soils, the boron forms and doses to be applied to the tea plant, and the boron yield-quality relationship *National Boron Research Institute (BOREN) Final Report*. Project No: 2012.30.06.20.007, 117, Ankara.

Taban, S., Turan, M. (2012). Plant Nutrition Management in Organic Agriculture Edt: Karaman MR. Gübretaş Guide Book Series: 2, Dumat Ofset, Ankara.

TEMA, (2022). Loss of organic matter. https://topraktema.org/kategoriler/yok-olan-toprak/nas%C4%B1l-yok-oluyor/organik-madde-kayb%C4%B1/ Date of access: 08.08.2022.

Vandez, V., Rao, S., Kholova, J., Krishnamurthy, L., Kashiwagi, J., Ratnakumar, P., Sharma, K.K., Bhatnagar- Mathur, P., Basu, P.S. (2008). Root research for drought tolerance in legumes: Quo vadis? *Journal of Food Legumes.* 21 (2): 77–85.

Varanasi, S.T. (2021). History of soil fertility and plant nutrition. *Just Agriculture*, 1(11):1–14.

Yılmaz, G.B., Atik, D., Sivri, N. (2017). Agricultural diffuse pollution and sustainability in Ergene basin. *European Journal of Sustainable Development Research* 2(1): 127-134.

CHAPTER 2

NUCELLAR EMBRYO DEVELOPMENT IN SOME CITRUS ROOTSTOCKS

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1. INTRODUCTION

Sexual reproduction is a dynamic process in flowering plants for producing seeds to move on heredity. But some angiosperms have evolved an alternative form of reproduction known as apomixis which is a form of asexual reproduction (Bicknell and Koltunow, 2004). Apomixis allows clonal seed production lowering production costs and enabling seed quality (Fei et al., 2019). At the same time it has been observed in more than 400 plant species including citrus and apple (Zhang et al., 2018). Some Citrus species form polyembryonic seeds by an apomictic process called nucellar embryony in which may embryos initiate directly from nucellar cells surrounding the sexual embryo sac (Koltunow et al., 1995).

Most of citrus genotypes are apomictic with some exceptions (Aleza et al., 2010) and this property allows the production of rootstocks by generating clones from nucellar tissue of mother plant (Almeida et al., 2018). At the same time, in a genotype that produces seeds by nucellar embryony, normal sexual reproduction can also occur (Kepiro and Roose, 2007). However, the presence of nucellar and zygotic embryos in the same seed indicates heterozygosity of embryonal population (Koltunow, 2000; Kishore et al., 2012) and unless the zygotic seedlings are identified accurately, non-true-to-type plants may lead to errors in nurseries (Hussain et al., 2011). Besides this, nucellar embryony hinders the formation of hybrid offspring and the progress of cross-breeding (Zhang et al., 2018) due to restriction of the zygotic embryo which have difficulty in surviving since they must compete for nutrients and space with the nucellar embryos (Perez-Torneo and Porras, 2008).

The tendency for production of nucellar embryos differs greatly among citrus species (Bowman et al., 1995) and ecology (Andrade –Rodriguez et al., 2004). In the present case, a genotype can produce several different types of seeds as;

seeds with one mature sexual embryo; seeds with one mature nucellar embryo; seeds with multiple mature nucellar embryos and seeds with one mature sexual and one or more mature nucellar embryos (Kepiro and Roose, 2007). Furthermore, although the presence of extra embryos from nucellar tissue gives rise to polyembryonic seeds in citrus, they sometimes may be produced by division of the zygotic embryo (Zhang et al., 2018) called cleavage embryony (Bacchi, 1943).

The polyembryony phenomenon was first reported by Leuwenhoek in 1719 with formation of two seedlings from one orange seed and firstly observed by Strasburger (1878) with histological analysis in *Citrus aurantium* L. giving the name "adventive embryos". The process is then progressed with Osawa in 1912; Frost in 1926 and Traub and Robinson in 1937 (Cook, 1907). Following these information numerous histological analyses were advanced for understanding this phenomenon in citrus (Bacchi, 1943; Esen and Soost, 1977; Kobayashi et al., 1979; Wilms et al., 1983; Wakana and Uemoto, 1987; 1988; Koltunow et al., 1995; Bowman et al., 1995; Carimi et al., 1998; Soares Filho et al., 2000; Andrade Rodrigues et al., 2004; Perez Torneo and Porras; 2008; Aleza et al., 2010; Kishore et al., 2012; Yıldız et al., 2013). These studies presented different and confusing information about the differentiation time of nucellar embryo initial cells (NEICs), pollination requirements, nutrition source, development processes, and presence of a zygotic embryo in each citrus species. However, there are still inexplicit confirmations in the formation of individual citrus genotypes, especially in rootstocks.

At present, all commercially cultivated citrus trees are grafted onto rootstock maintaining true-to-type varieties to overcome the adverse effects of abiotic and biotic stresses. Because of citrus rootstocks are selected to have a high percentage of nucellar seedlings, rootstock breeders have to obviate the restriction of the zygotic embryo by learning the nucellar and zygotic embryo formation of studied genotypes (Çimen and Yeşiloğlu, 2016). At the same time, the nurseries shall also acquaint to the cultivar to have true-to-type seedlings.

In this study, the nucellar embryo formation in important citrus rootstocks was aimed to investigate in terms of the relationship linking with pollination and fertilization by histological point of view. By this way nurseries and citrus rootstock breeders may obtain accurate and usable results for their works.

2. MATERIALS AND METHODS

Pollination treatments of this study were conducted in Citrus rootstock orchard of Alata Horticultural Research Institute (Mersin/Türkiye) from 2014 to 2016 and the histological analysis at Histology laboratory of Cukurova University (Adana/Türkiye) from 2014 to 2018.

In this study, highly recommended apomictic citrus rootstocks 'Carrizo citrange', 'Cleopatra mandarin', and 'Volkameriana' were examined. For pollination studies, *Poncirus trifolata* 'Yerli' was used as a pollenizer. The used rootstocks are highly recommended worldwide because of their superior rootstock characteristics. In this view, 'Carrizo citrange' is immune to Citrus Tristeza Virus (CTV), tolerant to nematodes and *Phytophtora*; 'Cleopatra mandarin' is highly tolerant to soil salinity and 'Volkameriana' is tolerant to CTV and well adapted to calcareous soil (Dambier et al., 2011). For that reason, they are highly recommended for both breeding programs and rootstock producers.

Flower bud sampling

Flower buds in different sizes are collected to investigate ovule development and nucellar embryo formation and fixed immediately in FPA 70 (formaldehyde-propionic acid-alcohol) (Gerlach, 1984). Each size of flower bud was also marked on the tree with a permanent dye and observed their anthesis days to detect their duration before anthesis.

Pollination treatments and sample collection

Four hundred unopened flower buds of each rootstock were emasculated with forceps carefully to avoid any injury to the pistil just before their opening in 2014 and 2015. All previously opened flowers and small immature buds were removed. Emasculated flowers were isolated with cotton bags to avoid free pollination. One day later, at anthesis, 300 of the emasculated flowers were cross-pollinated with *Poncirus trifoliata* 'Yerli' pollens by hand using a small brush. The rest of the emasculated flowers remained unpollinated. All pollinated and unpollinated flowers were isolated with cotton bags again immediately. The pollination day of the flowers was accepted as the beginning of anthesis, and the day count was started after this process. The pollinated flower samples were collected in 1-day intervals from 1 DAA (the day after anthesis) to 5 DAA; 2 days intervals from 7 DAA to 15 DAA; 7 days intervals from 15 DAA to 85 DAA and 15 days intervals from 85 DAA until harvest and fixed with FPA 70 immediately. The unpollinated flowers are collected on the same days with cross-pollinated flowers until all of them drop. A deep and narrow incision was made in the large ovaries and seeds with a razor blade to facilitate rapid penetration of the fixative.

Pollen tube growth

Pollen tube growth rates of ‘Carrizo citrange’, ‘Cleopatra mandarin’, and ‘Volkameriana’ were evaluated from cross-pollinated flowers from 1 DAA to 15 DAA in 2 days intervals. Five flowers per each sampling date were examined for pollen tube growth rate analysis. Pollen tubes in the stigma and style, as well as ovules, were monitored on squash preparations of pistils, previously softened in 8N sodium hydroxide for 5-7 h, stained with 0.1% aniline blue in 0.1 N K_3PO_4 (Kho and Baer, 1970; Preil, 1970, Karabıyık, 2022) and observed under a fluorescence microscope (Olympus BX51, Tokyo, Japan) equipped with a U-MWU filter (Olympus, Tokyo, Japan). Pollen tube growth was determined as the percentage of the style traversed by the longest pollen tube in each pistil by a digital micrograph system (Olympus DP72 camera, Tokyo, Japan).

Nucellar embryo development

The paraffin sectioning method was used for embryo formation analysis. Five samples from all fixed ovaries of flower buds and pollinated and unpollinated ovaries were dehydrated in a series of ethanol and tert-butanol solutions and embedded in paraffin (Johansen, 1940). Then the samples were blocked on a wood block and sectioned with a rotary microtome (Leica RM2135, Leica, Wetzlar, Germany) at 10µ thickness. All preparations were stained with 0.125% hematoxylin buffered with $KMnO_4$ and mounted in Entellan (Eti, 1987; Karabıyık et al., 2018). Samples were observed with fluorescence microscope (Olympus BX51, Tokyo, Japan) equipped with a U-MWU filter (Olympus, Tokyo, Japan) and photos were obtained by a digital micrograph system (Olympus DP72, Tokyo, Japan).

3. RESULTS

Flower bud development

The ovule development in flower buds was not uniform and differed between rootstocks. The ovule development in different flower bud stages was shown in Figure 1. In 10 days before anthesis (DBA) all rootstocks had ovule primordia present in most of their carpels. The archesporial cell was present simultaneously with integument formation (Figure 1A) and first seen in ‘Cleopatra mandarin’ at 9 DBA while it is 7 DBA in ‘Volakameriana’ and 6 DBA in ‘Carrizo citrange’. As far as archesporial cell formation, it divided to form the tapetal cell and the megasporocyte. Subsequently, the nucellus was started to form by tapetal cell division while the megasporocyte waited a while before division (Figure 1B). In this stage, the integuments also initiated and quickly covered half of the nucellus. Then, the megasporocyte underwent meiosis forming firstly 2 megaspores and then 4 megaspores (Figure 1C) which then called sister megaspores. Three of these sister megaspores, near the micropyle, degenerated expeditiously leaving only the lowermost one in order to form functional megaspore (Figure 1D). After that, nucellus of the functional megaspore underwent first mitotic division to form 2-nucleate embryo sac (Figure 1E) which started firstly at 3 DBA in ‘Carrizo citrange’, 3-4 DBA in ‘Cleopatra mandarin’ and 3-4 DBA in ‘Volkameriana’. The first division was followed by second mitotic division to form 4-nucleate and third mitotic division to form 8-nucleate embryo sac (Figure 1F) where the fertilization will take place. At anthesis, while ‘Cleopatra mandarin’ and ‘Volkameriana’ had 8-nucleate embryo sacs in most of their ovules, ‘Carrizo citrange’ had the capacity to differentiate as far as 4-nucleate stage.

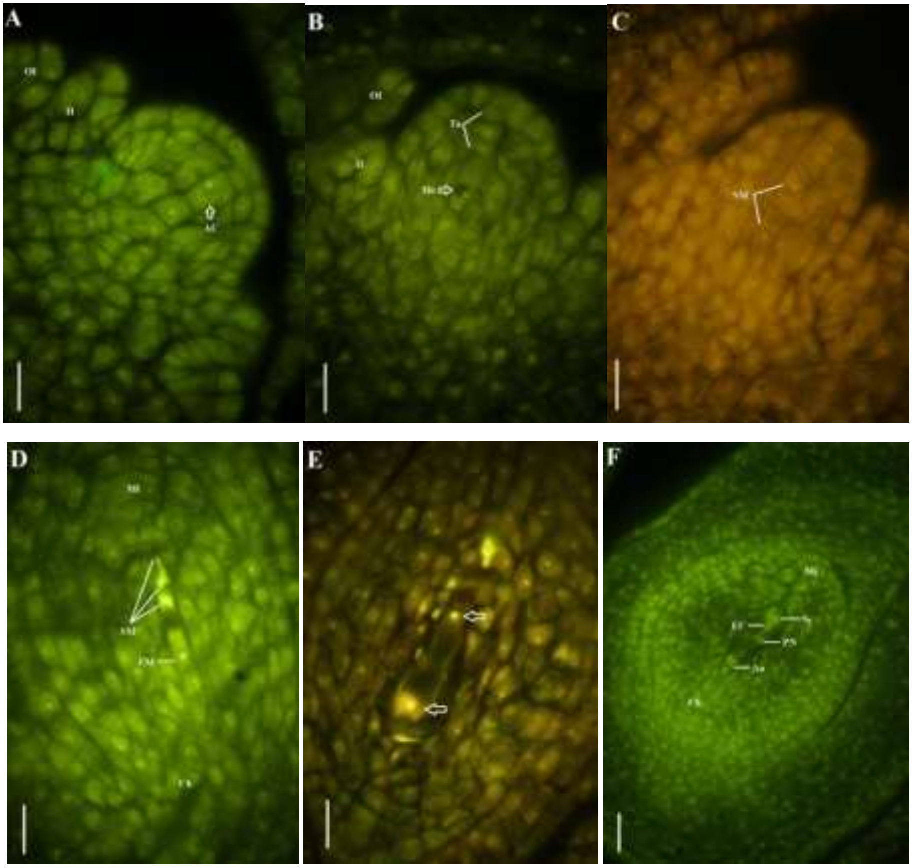

Figure 1. Ovule development in different flower bud stages
A. Archesporial cell present and the inner and outer integuments are initiated. Scale bar: 10µm; B. Formation of the megasporocyte and the tapetal division (Scale bar: 10µm); C. Meiosis stage of megasporocyte forming sister megaspores (Scale bar: 10µm); D. Degeneration of 3 sister megaspores leaving functional megaspore (Scale bar: 10µm). E. Two nuclei (white arrows) of binucleate embryo sac (Scale bar: 20µm); F. Eight nucleate embryo sac (Scale bar: 20µm).
Abbreviations: AC, Archesporial cell; An, Antipodals; Ch; Chalaza; EC, Egg cell; FM: Functional megaspore; II, Inner integument; Mi, Micropyle; Ms, Megasporocyte; OI, Outer integument; PC, Polar nuclei; SM, Sister megaspores; S, Synergids; Ta, Tapetal Cells.

The synchronization of ovule formation was also different among rootstocks. In 'Carrizo citrange' and 'Cleopatra mandarin', the ovule development synchronized but 'Volkameriana' had different ovule stages from megasporocyte to 8-nucleate mature embryo sac in the same ovarium.

Formation of nucellar embryo initial cells (NEICs) in flower buds

In flower bud stage, the ovules of 'Carrizo citrange' have no distinct nucellar embryo initial cells (NEICs) and the ovules continued to their normal development. In 'Cleopatra mandarin' and 'Volkameriana' some of the nucellus cells differentiated but they were not plentiful and seen only in two or three ovules at around 8-nucleate stage.

Pollen tube growth

In order to characterize the fertilization time, pollen tube growth rate was determined for cross pollination treatments made in 2014 and 2015 growing periods. After germination, pollen tubes grew between the parenchyma cells of the stigmoid tissue reaching the stylar canals in first 3 days (Figure 2A) for all studied cultivars. Then, the pollen tubes proceeded expeditiously through the style (Figure 2B) and penetrated to the ovule (Figure 2C) to fertilize the egg cell (Figure 2D). The pollen tube growth rates were not different for both years. In 'Carrizo citrange', pollen tubes penetrated to the ovule at 9 days after anthesis (DAA). For 'Cleopatra mandarin' this period was 5 DAA and it was 9 DAA for 'Volkameriana'.

Formation and development of NEICs

In ovules of studied rootstocks all NEICs were observed in the individual cells of nucellar tissue. The NEICs could easily be distinguished from other nucellus cells by their dense cytoplasm, larger cell and nuclei with greater affinity for staining (Figure 3A). The microscope observations of 'Carrizo citrange' showed that the initial cells were first seen at 11 DAA (Figure 3B). In 'Cleopatra mandarin' this stage was 5 DAA (Figure 3C) while it is 4 DAA in 'Volkameriana' (Figure 3D). After pollen tube penetration to the ovule, the zygote formed at the micropylar end of the embryo sac and the endosperm started to form with a haustorium towards the chalazal end (Figure 3E).

The NEICs were first started to form in the nucellar cell layers surrounding the chalazal half of the embryo sac both in ‘Carrizo citrange’ and ‘Cleopatra mandarin’ while the NEICs of ‘Volkameriana’ differentiated in a few layers away from embryo sac closer to inner integument. The highest identified NEIC numbers were also different between cultivars as 13, 18 and 24 for ‘Volkameriana’, ‘Cleopatra mandarin’ and ‘Carrizo citrange’, respectively.

In the first stages of NEIC development, the nucellar cells surrounding NEICs started to degenerate showing that NEICs used up nucellus for all studied rootstocks (Figure 3F). From now on, the NEICs started to divide in 29 DAA in ‘Carrizo citrange’ and before the division of zygote; 57 DAA in ‘Cleopatra mandarin’ simultaneously with the zygote. This stage could not determine in ‘Volkameriana’ exactly but it is estimated between 50 70 DAA, after the division of zygote in 36 DAA.

Before the initiation of divisions, the NEICs enlarged and rounded (Figure 3F) and after division, young embryos took globular shape and used nucellus for nutrition and for entrancing into the embryo sac. The nucellar embryos located in the micropylar end of the ovule because the endosperm was well developed at embryo division stage and already took its place in the chalazal part of the ovule. After the nucellar embryo entrance to the endosperm, nucellar embryos started to use up endosperm and accelerated their growth.

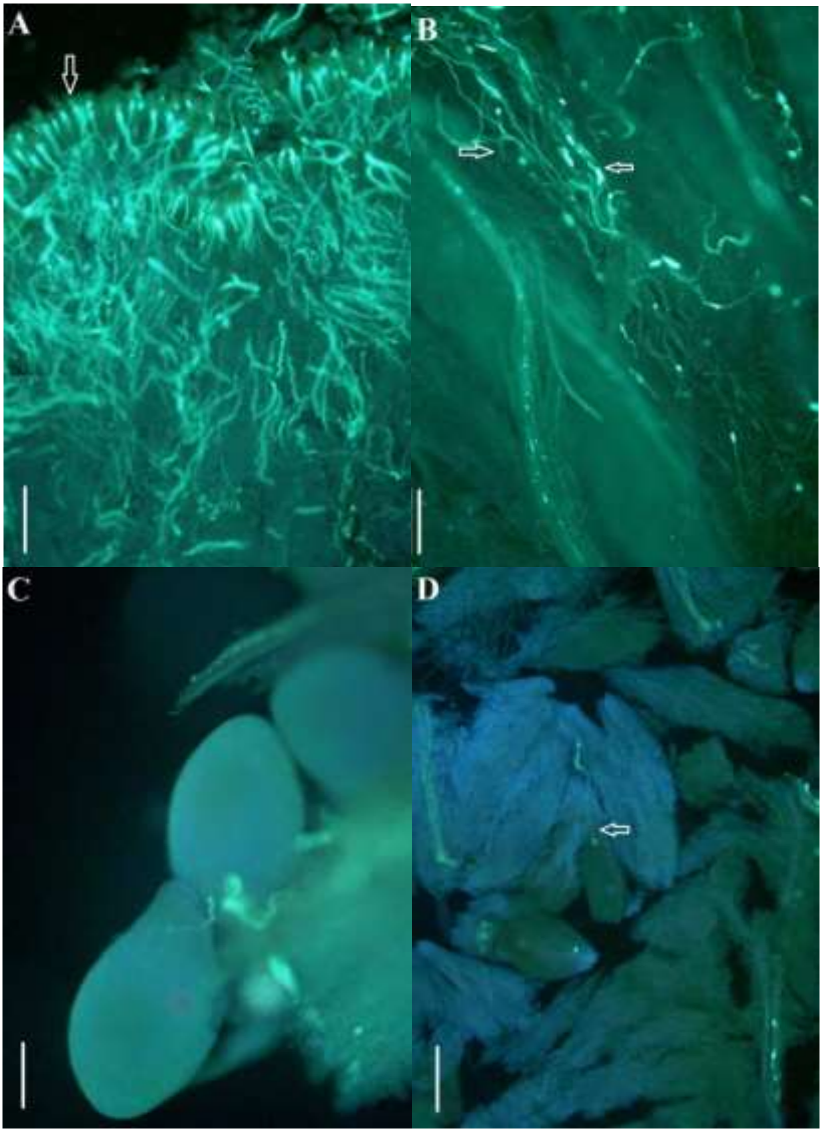

Figure 2. Pollen tube growth in citrus rootstocks
A. Pollens germinated in the stigma (white arrow) and pollen tube start to elongate throughout the stigmoid tissue (Scale bar: 100µ). B. Pollen tube growing towards the ovary inside the stylar canals (white arrow) (Scale bar: 100µ). C. Pollen tube penetration to the ovule from micropyle (Scale bar: 50µ). D. Pollen tube penetration (white arrow) to the embryo sac in squashed ovule (Scale bar: 50µ)

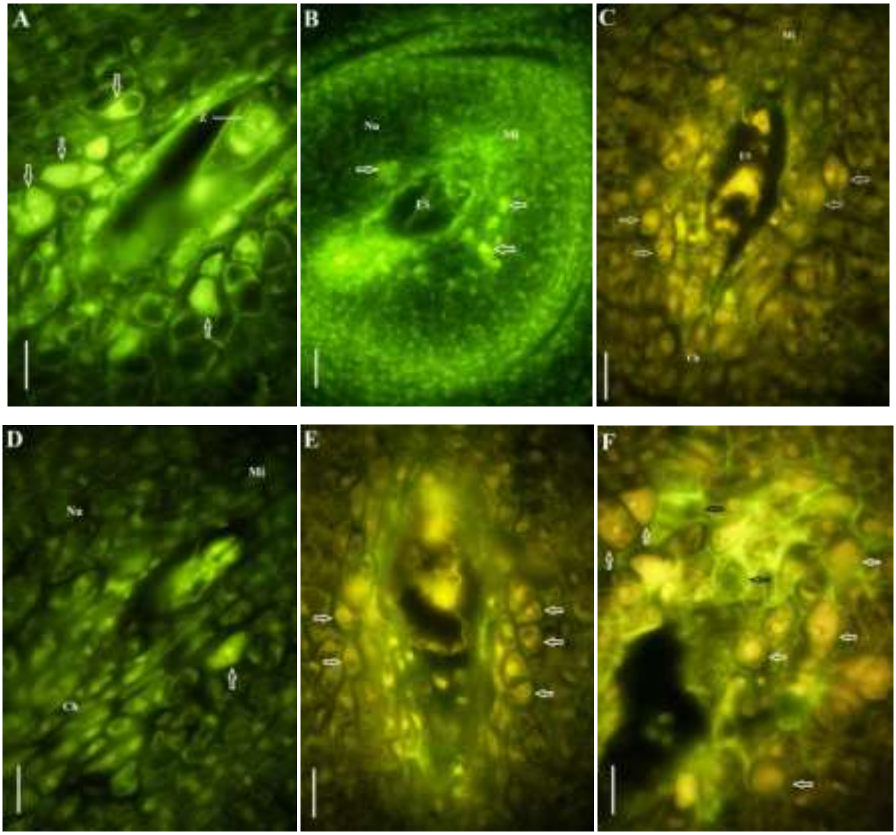

Figure 3. Formation of nucellar embryo initial cells
A. Dense staining nucellus cells inside the ovule (white arrows) (Scale bar: 10µm). B. NEICs (white arrows) seen at 11 DAA in the vicinity of embryo sac in 'Carrizo citrange' (Scale bar: 20µm). C. NEICs (white arrows) seen at 5 DAA towards the chalasal end in 'Cleopatra mandarin' (Scale bar: 10µm). D. NEICs (white arrows) seen in 4 DAA in 'Volkameriana' (Scale bar: 10µm). E. Endosperm formation (Scale bar= 10µm). F. Degeneration of nucellar cells (black arrows) surrounding NEICs (white arrows) (Scale bar: 10µm).
Abbreviations: Ch, Chalaza; ES, Embryo sac; Mi, Micropyle; Nu, Nucellus; Z, Zygote

In 'Carrizo citrange', lots of NEICs started to divide before the division of the zygote and entered into the embryo sac for using endosperm (Figure 4A). However, in 'Cleopatra mandarin' a high number of NEICs divided simultaneously with zygote and all the embryos started to form their suspensor at first. Then, all of the embryos together entered into the embryo sac (Figure

4B). This process for 'Volkameriana' was same as 'Carrizo citrange' but this time, lower in number as 2 or 3 NEICs. After this stage, the nucellar embryos at all stages, from globular until cotiledonary, usually crowded within the same seed (Figure 4C). These different division properties caused differences in zygotic embryo development. Division of NEICs together with zygotic embryo caused a restriction in zygotic embryo development in 'Carrizo citrange' (Figure 4D) and 'Cleopatra mandarin' (Figure 4E) while the zygotic embryo in 'Volkameriana' could easily form in most of the ovules (Figure 4F).

Nucellar embryo development progressed until seed formation. In 'Carrizo citrange' embryos filled the seed coat at 85 DAA and the number of nucellar embryos at this stage was 7 in maximum. However, in 'Cleopatra mandarin' embryos could not fill seed until 170 DAA with lots of crowded nucellar embryos inside the seed coat. This process was 115 DAA for 'Volkameriana' with only 2 to 4 embryos in general.

Nucellar embryo development in unfertilized flowers

Unfertilized flowers were also examined until all of the flowers abscissed. Although the nucellus tissue and the embryo sac degenerated expeditiously in 'Carrizo citrange' and 'Volkameriana' (Figure 5A); in 'Cleopatra mandarin', the nucellar tissue was in normal appearance and the NEICs started to differentiate in 5 DAA (Figure 5B). However, these NEICs also degenerated until 9 DAA (Figure 5C).

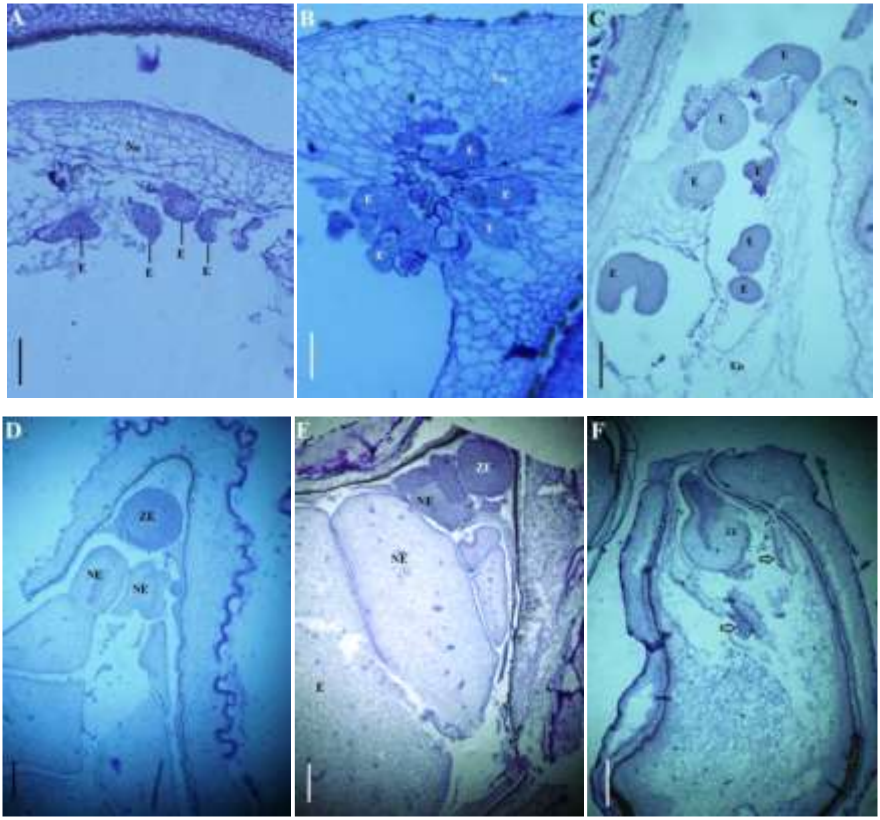

Figure 4. Nucellar embryo development in citrus rootstocks
A. Globular embryos entering embryo sac in ‘Carrizo citrange’ (Scale bar: 50µm). B. Suspensor formation of zygotic and nucellar embryos in ‘Cleopatra mandarin’ (Scale bar: 50µm). C. Different phases of embryo developing inside the endosperm in the same embryo sac (Scale bar: 100µm). D. Restricted zygotic embryo and developing nucellar embryos in ‘Carrizo citrange’ (Scale bar: 200µm). E. Mature seed with a restricted zygotic embryo (Scale bar: 200µm). F. Zygotic and nucellar embryo (black arrows) development in ‘Volkameriana’ (Scale bar: 200µm). Abbreviations: E, Embryo; NE; Nucellar embryo; Nu, Nucellus; ZE, Zygotic embryo

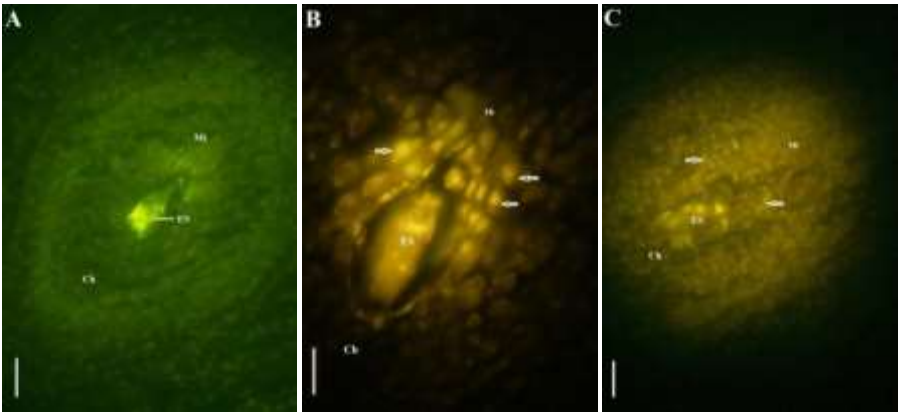

Figure 5. Nucellar embryo development in unfertilized ovules
A. Degeneration of nucellar tissue and the embryo sac in ‘Carrizo citrange’ ovules at 8 DAA (Scale bar: 20µm). B. NEIC formation (white arrows) in unfertilized ovules of ‘Cleopatra mandarin’ in 5 DAA with the normally developed embryo sac (Scale bar: 10µm). C. Degenerated NEICs (white arrows), nucellar tissue and embryo sac in ‘Cleopatra mandarin’ at 9 DAA (Scale bar: 20µm)
Abbreviations: Ch, Chalasa; ES, Embryo sac; Mi, Micropyle

4. DISCUSSION

This study reports the nucellar embryo development in terms of histological background for citrus rootstocks ‘Carrizo citrange’, ‘Cleopatra mandarin’ and ‘Volkameriana’. At the same time, the role of pollination and fertilization on nucellar embryo initiation, development and seed formation were also analyzed in histological perspective. The study showed that the nucellar embryo formation and development in each rootstock have different structures from flower bud stage until mature embryo formation for the first time.

Eight-nucleate embryo sac, which called megagametophyte, matures a few days before or by the day of anthesis in general. In this research, ‘Cleopatra mandarin’ and ‘Volkameriana’ had matured megagametophytes in most of their ovules while ‘Carrizo citrange’ could only complete first or second mitotic division forming two or four-nucleate stages. Bacchi (1943) have been reported that there can be one-, two- or four-nucleate stages in megagametophytes of opened flowers in some instances. Similarly, Koltunow et al. (1995) found in *Citrus sinensis* 'Valencia' that the megagametophyte was in two- or four-nucleate stages and formed the mature megagametophyte a few days after anthesis. In this study, 'Volkameriana' showed unsynchronous ovule development phases in the same ovary which was described by Bacchi (1943) before.

Throughout the flower bud stages of studied rootstocks, there were no clearly observed differentiated cells. Besides this, in the ovules of 'Cleopatra mandarin' and 'Volkameriana', some cells give rise to have denser fluorescent appearance which is thought to be associated with NEIC formation. However, further supplementary studies are required in order to determine that they are NEIC or not. In fact, Koltunow et al. (1995) have been reported in *Citrus sinensis* 'Valencia' that since the NEICs cannot be detected in flower bud stage by histological analysis, there were embryo formation in *in vitro* culture conditions of flower buds. Kobayashi et al. (1979) also showed embryo formation in flower bud stage of polyembryonic cultivars but not in monoembryonics. Moreover, Şimşek et al. (2019) reported that polyembryonic 'Orlando tangelo' have been showed differently expressed genes from monoembryonic cultivars and this time was coincides before histological differentiation of NEICs which was 3 DAA for 'Orlando tangelo'. So, these findings suggest that there are endogenous physiological preparations in nucellus tissue to form NEIC before they become apparent histologically.

NEICs were first seen in 11 DAA in 'Carrizo citrange', 5 DAA in 'Cleopatra mandarin' and 4 DAA in 'Volkameraina' with their denser cytoplasm, larger cell with a larger nuclei and greater affinity for staining than normal nucellar cells. These cells were described as this way by Wilms et al. (1983); Wakana and Uemoto, (1987) and Koltunow et al. (1995) before and the NEICs were also linked with an undivided zygote by Zhang et al. (2018). The detailed studies by Wilms et al. (1983) have been showed that there is a distinct layer between the plasma membrane and the primary cell wall which has a granular, heterogenic and electron density material and this differs them from the other nucellus cells.

One of the most important questions in nucellar embryo formation is pollination requirement. In relation to that, three different suggestions were determined in this study. Firstly, NEICs of 'Cleopatra mandarin' gave rise after anthesis both in pollinated and unpollinated pistils nearly at the same stages indicating NEICs form autonomously independent from pollination. The other finding was in 'Volkameriana'. In this rootstock, the NEICs did not form in unpollinated pistils while they formed in pollinated ones before pollen tube penetration to the ovule. This reveals the second suggestion as pollination is necessary for NEIC differentiation. At last, in 'Carrizo citrange' NEICs could only form after fertilization of the ovule suggesting pollination is not only the necessity to form NEICs, but also fertilization is absolutely essential. Recent studies have also been reported different results like pollination is not required and NEICs can form without pollination (Kobayashi et al., 1979; Wakana and Uemoto, 1987; 1988); they can form after pollination but before fertilization (Şimşek et al., 2019) and pollination is strictly necessary for NEIC formation and development (Mendes Rodrigues et al., 2005; Karabıyık and Eti, 2020). Such decisions had also been described in this study demonstrating the differences for each progeny.

Although used cultivars have differences in pollination requirement for NEIC formation, they are dependent on fertilization and consequential endosperm formation for development of nucellar embryos as reported by almost all researchers. At the same time, all of the NEICs were start to use up the nucellus cells in their vicinity at first until reaching to the endosperm, which concurrently continues to develop inside the embryo sac. Although the NEICs firstly formed in the vicinity of embryo sac; before reaching endosperm they were observed at the micropylar end. By this time, the endosperm also continued to develop towards the chalasal end. In most of the studies, the NEICs were generally suggested to develop in micropylar end (Esen and Soost, 1977; Koltunow et al., 1995) and rarely develop at chalasa (Wakana and Uemoto, 1987). Histological studies showed that this may be originated from the lack of space in the chalasal end and for that reason; the NEICs were obligated to develop towards the micropylar end. This can be supported by the findings of Wakana and Uemoto (1987) and Koltunow et al. (1995) in unpollinated seeds that there are nucellar embryos in the chalasa. The unpollinated and undeveloped seeds have no endosperm and this provides the NEICs to develop in where they start to form. The undeveloped seeds were not analysed in this study but the observational data gives accuracy to this suggestion.

By the NEICs reached to the endosperm, the nucellar embryos developed quickly passing globular, heart shaped, torpedo and cotiledonary phases, respectively. Then, the formed embryos occupied the space previously filled by the endosperm. At this stage, the division time of the nucellar embryos was important and this process is started before zygotic embryo division in 'Carrizo citrange', with the zygotic embryo division in 'Cleopatra mandarin' and after zygotic embryo division in 'Volkameriana'. These processes give rise to zygotic embryo restriction in 'Carrizo citrange' and 'Cleopatra mandarin' while zygotic 'Volkameriana' embryos can develop easily. Carimi et al. (1998) and Perez-Tornero and Porras (2008) reported that, it was possible to identify nucellar and zygotic embryos at the beginning. However, with the entrance of NEICs to the embryo sac, the quick embryo development caused the zygotic embryos to be restricted and not be distinguished from the adventitious ones. In previous studies, the zygotic seedling formation ratios in ‘Volkameriana’ after cross pollination studies were reported to be higher than other cultivars (Garcia et al., 1999; Kepiro and Roose, 2007; Gora et al., 2018). The beginning of zygotic embryo division before nucellar ones clearly explained the reason of zygotic seedling formation in ‘Volkameriana’. In the contrary, low zygotic seedling occurrence in ‘Cleopatra mandarin’ was also reported by Dambier et al. (2011) before and again this study showed the process of restriction in this rootstock caused by development of nucellar and zygotic embryos from the same place and stage.

Fruit development does not occur from emasculated flowers. This suggests that double fertilization for endosperm formation is a necessary stimulus for the development of both zygotic and nucellar embryos in the studied rootstocks. Koltunow et al. (1995) have already been reported this opinion in non parthenocarpic *Citrus sinensis* ‘Valencia’. However, in parthenocarpic *Citrus sinenesis* ‘Washington navel’, Wakana and Uemoto (1988) and Karabıyık et al. (2017) have been reported that there may form seeds in some conditions suggesting these seeds are apomictic. At the same time, most apomictics are pseudogamous (Koltunow, 1993) and require fertilization of the polar nuclei for endosperm development (Maheshwari, 1950). In some cases, endosperm develops autonomously (Rojek et al., 2015) which cannot be seen in citrus (Koltunow, 1993).

Recent studies also showed that the zygotic embryos could be rescued *in vitro* before their nutrient is restricted by nucellar embryos at the globular or heart stage of the zygotic embryos. In this study, this stage can be suggested as about 55-65 DAA for ‘Carrizo citrange’, 65-75 DAA for ‘Cleopatra mandarin’ and

65-80 DAA for ‘Volkameriana’. This type of study was made by Carimi et al. (1998) in *in vitro* conditions and suggested after 105 DAA for *Citrus aurantium*. In another study conducted in *Citrus lemon*, 50 - 85 DAA were suitable for embryo rescue while at 165 DAA embryos were too crowded to separate from each other (Perez Torneo and Porras, 2008). Karabıyık and Eti (2020) have also suggested this process to be 65-75 DAA in *Citrus aurantium* ‘Yerli’. In this study, the division and development type of ‘Cleopatra mandarin’ may not allow this type of embryo rescue because of the nucellar embryos and the zygotic embryo started to develop at the same place and stage. Moreover, all of the nucellar embryos first developed their suspensors towards the suspensor of the zygotic embryo showing a very crowded embryo existence in the micropylar end. This type of nucellar embryo development was the first to be reported in the literature and may be the reason for high seedling survival but very low zygotic seedling formation ratio which is reported by Caruso et al. (2014) in their cross breeding studies on ‘Cleopatra mandarin’ before.

Embryos, regardless of being zygotic or nucellar, fill the seed coat at different rates and different processes. Embryos of ‘Carrizo citrange’ were the first to fill its seed coat and became mature earlier than other rootstocks. Later, the embryos of ‘Volkameriana’ and ‘Cleopatra mandarin’ filled seed coats and ripened their fruits. The embryo number inside the seeds also differed among rootstocks, and this is related with the NEIC number at the beginning. The high number of nucellar embryos causes the degeneration of cotyledons. This type of embryo development was also reported by Bowman et al. (1995) and Kishore et al. (2012) in mature citrus seeds and by Mendes-Rodrigues et al. (2005) in *Eriotheca pubescens* as more than one embryo generally restricts embryo development.

It is clear in the paper that the histological stages from flower bud to seed development can differ between genotypes in terms of nucellar embryony. Therefore, the formation and development of nucellar embryony for individual citrus genotypes should be researched. Fei et al. (2019) and Şimşek et al. (2019) have reported that to clarify the mechanism of apomixis, simultaneous examinations of both genetic and cytological evidence are required. Moreover, cytological observation should be used first to identify the key periods of embryogenic development.

5. CONCLUSION

The results of this study showed that the complexity of nucellar embryony and apomixis is derived from the variations between different genotypes in terms of formation time, formation places, and development of NEICs. This result impresses citrus nurseries and breeders to learn the apomictic properties of the studied cultivar or genotype before planning to have more accurate results in a shorter time.

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REFERENCES

Aleza, P., Juarez, J., Ollitrault, P., Navarro, L. (2010). Polyembryony in non-apomictic citrus genotypes. *Annals of Botany* 106: 533-545.

Almeida, L.A.D.H., Santana-Vieira, D.D.S., Santos, N.D.A., et al. (2018). Water deficit increases the frequency of hybrid citrus with polyembryonic female parents. *Crop Breeding and Applied Biotechnology* 18(1): 47-54.

Andrade-Rodriguez, M., Villegas-Moner, A., Carrillo-Castaneda, G., Garcia-Velazquez, A. (2004). Polyembryony and identification of Volkamerian lemon zygotic and nucellar seedlings using RAPD. *Pesquisa Agropecuária Brasileira* 39(6): 551-559.

Bacchi, O. (1943). Cytological observations in Citrus: III. Megasporogenesis, fertilization and polyembryony. *Botanical gazette* 105(2): 221-225.

Bicknell, R.A., Koltunow, A.M. (2004). Understanding apomixis: Recent advances and remaining conundrums. *The Plant Cell* 16: 228-245.

Bowman, K.D., Gmitter, F.G., Hu, X. (1995). Relationship of seed size and shape with polyembryony and the zygotic or nucellar origin of *Citrus* spp. seedlings. *Hortscience* 30(6): 1279-1282.

Carimi, F., Pasquale, F., Puglia, A.M. (1998). *In vitro* rescue of zygotic embryos of sour orange, *Citrus aurantium* L., and their detection based on RFLP analysis. *Plant Breeding* 117: 261-266.

Caruso, M., Distefano, G., Paolo, D.P., La Malfa, S., Russo, G., Gentile, A., Recupero, G.R. (2014). High resolution melting analysis for early identification of citrus hybrids: A reliable tool to overcome the limitations of morphological markers and assist rootstock breeding. *Scientia Horticulturae* 180: 199-206.

Çimen, B., Yeşiloğlu, T. (2016). Rootstock breeding for abiotic stress tolerance in citrus. Abiotic and Biotic Stress in Plants-Recent Advances and Future Perspectives. Intech Open, London, UK.

Cook, M.T. (1907). Notes on polyembryony. *Torrey Botanical Society* 7(6): 113-117.

Dambier, D., Benyahia, H., Pensabene-Bellavia, G., Aka Kaçar, Y., Froelicher, Y., Belfalah, Z., Ollitrault, P. (2011). Somatic hybridization for citrus rootstock breeding: An effective tool to solve some important issues of the Mediterranean citrus industry. *Plant Cell Reports* 30: 883-900.

Esen, A., Soost, R.K. (1977). Adventive embryogenesis in Citrus and its relation to pollination and fertilization. *American Journal of Botany* 64(6): 607-614

Eti, S. (1987). *Über das pollen schlauchwachstum und die entwicklung der samenlagen in beziehung zum fruchtansatz und zur frucht qualitaet bei der manderinensorte "Clementine" (Citrus reticulata Blanco).* (PhD Thesis), Dissertation University of Hohenheim, Germany

Fei, X., Shi, J., Liu, Y., Niu, J., Wei, A. (2019). The steps from sexual reproduction to apomixis. *Planta* 219: 1715-1730.

Garcia, R., Asins, M.J., Forner, J., Carbonell, E.A. (1999). Genetic analysis of apomixis in Citrus and Poncirus by molecular markers. *Theoretical and Applied Genetics* 9: 511-518.

Gerlach, D. (1984). Botanische Mikrotechnik. George Thieme Verlag, Stuttgart-New York

Gora, J.S., Kumar, R., Sharma, B.D., Ram, C., Kumar, K. (2018). Determination of morphological diversity for seed and seedling characteristics in citrus rootstocks. *International Journal of Chemical Studies* 6(6): 2921-2926.

Hussain, S., Curk, F., Ollitrault, P., Morillon, R., Luro, F. (2011). Facultative apomixis and chromosome doubling are sources of heterogeneity in citrus rootstock trials: Impact on clementine production and breeding selection. *Scientia Horticulturae* 130(4): 815-819

Johansen, D.A. (1940). Plant microtechnique. McGraw-Hill Book Company, Inc: London; 530p.

Karabıyık, Ş. (2022). Effects of temperature on pollen viability and in vivo pollen tube growth in *Citrus sinensis*. *Journal of Applied Botany and Food Quality* 95: 100-104.

Karabıyık, Ş., Eti, S. (2020). Nucellar embryony and formation mechanism in Sour Orange. *Yuzuncu Yil University Journal of Agricultural Science*, 30(4), 761-771.

Karabıyık, Ş., Eti, S., Yılmaz, B., Sağır, F.S. (2017). Effects of pollination on fruit set and some fruit quality properties of navel group orange cultivars. *Alatarım* 16(1): 11-18

Karabıyık, Ş., Gündeşli, M.A., Kafkas, S., Güney, M., Zarıfıkhosroshahı, M., Kafkas, N.E. (2018). Detection of bud abscission of pistachio via histological analysis. *Acta Horticulturae* 1219: 193-198.

Kepiro, J.L., Roose, M.L. (2007). Nucellar Embryony. Citrus Genetics, Breeding and Biotechnology, CAB International. USA.

Kho, Y.O., Baer, J. (1970). Die fluoreszenmikroskopie in der botanischenforschung. *Zeiss Information* 18: 54-57.

Kishore, K., Monika, N., Rinchen, D., Lepcha, B. and Pandey, B. (2012). Polyembryony and seedling emergence traits in apomictic Citrus. *Scientia Horticulturae* 138: 101-107.

Kobayashi, S., Ieda, I., Nakatani, M. (1979). Studies on the nucellar embryogenesis in Citrus. II. Formation of the primordium cell of the nucellar embryo in the ovule of the flower bud and its meristematic activity. *Journal of Japanese Society for Horticultural Science* 48:179-185.

Koltunow, A.M. (1993). Apomixis: Embryo sacs and embryos formed without meiosis or fertilization in ovules. *Plant Cell* 5: 1425-1437.

Koltunow, A.M. (2000). The genetic and molecular analysis of apomixis in the model plant Hieracium. *Acta Biologica Cracoviensia, Series Botanica* 42: 61-72.

Koltunow, A.M., Soltys, K., Nito, N., McClure, S. (1995). Anther, ovule, seed and nucellar embryo development in *Citrus sinensis* cv. Valencia. *Canadian Journal of Botany* 73: 1567-1582.

Maheswari, O. (1950). An introduction to the embryology of angiosperms. Mgraw Hill, New York, London.

Mendes-Rodrigues, C., Carmo-Oliveira, R., Talavera, S., Arista, M., Ortiz, P.L., Oliveira, P.E. (2005). Polyembryony and apomixis in *Eriotheca pubescens* (Malvaceae-Bombacoideae). *Plant Biology* 7: 533-540.

Perez-Tornero, O., Porras, I. (2008). Assessment of polyembryony in lemon: rescue and *in vitro* culture of immature embryos. *Plant Cell Tissue and Organ Culture* 93: 173-180

Preil, W. (1970). Fluoreszen mikroskopische beobachtung des wachstums von pollen schlauchen in griffel- und frucht knotengewebe. *Zeiss Information* 18: 24-25

Rojek, J., Pawelko, L., Kapusta, M., Naczk, A., Bohdanowicz, J. (2015). Exogenous steroid hormones stimulate full development of autonomous endosperm in *Arabidopsis thaliana*. *Acta Societatis Botanicorum Poloniae* 84(2): 287-301.

Şimşek, Ö., Dönmez, D., Eti, S., Yeşiloğlu, T., Aka Kaçar, Y. (2019). Comparative transcriptome sequencing to determine genes related to the nucellarembrony mechanism in citrus. *Turkish Journal of Agriculture and Forestry* 43: 58-68.

Soares Filho, W.D.S., Moreira, C.D.S., Cunha, M.A.P.D., Cunha Sobrinho, A.P.D., Passos, O.S. (2000). Polyembryony and hybrids frequency in *Citrus* spp. *Pesquisa Agropecuaria Brasileira* 35(4): 857-864.

Strasburger, E. (1878). Über Polyembryonie. *Jenaische Zeitschriftfür Naturwissenchaft* 12: 647–667.

Wakana, A., Uemoto, S. (1987). Adventive embryogenesis in Citrus. I. Occurence of adventive embryos without pollination or fertilization. *American Journal of Botany* 74: 517-530.

Wakana, A., Uemoto, S. (1988). Adventive embryogenesis in *Citrus* (*Rutaceae*). II. Postfertilization development. *American Journal of Botany* 75: 1031-1047.

Wilms, H.J., Went, J.L., Cresti, M., Ciampolini, F. (1983). Adventive Embriyogenesis in Citrus. *Caryologia* 36(1): 65-78.

Yıldız, E., Kaplankıran, M., Demirkeser, H., Uzun, A., Toplu, C. (2013). Identification of zygotic and nucellar individuals produced from several citrus crosses using SSRs markers. *Notulae Botanica Cluj-Napoca* 41(2): 478-484.

Zhang, S., Liang, M., Wang, N., Xu, Q., Deng, X., Chai, L. (2018). Reproduction in woody perennial citrus: An update on nucellar embryony and self-incompatibility. *Plant Reproduction* 31(1): 43-57.

CHAPTER 3

PRODUCTION OF BLACK TABLE OLIVES IN MICROWAVE DRYER WITH DIFFERENT PRETREATMENTS

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1. INTRODUCTION

Table olives are one of the oldest fermented plant origin foods in the Mediterranean region. Fermented table olives represent an important healthy food in the Mediterranean diet due to their high content of bioactive and health-promoting compounds as well as their economic impact (Perpetuini et al. 2020). Table olives are processed to reach an acceptable level of bitterness, improve sensory properties and ensure consumption safety (IOOC, 2004). Three main processing technologies are used worldwide; "Spanish style" (immersion in diluted caustic solutions, followed by washing step to remove excess caustic, and finally partial fermentation in brine), "California style" (brine-treated black olives), and "natural olives" (untreated produced by natural fermentation) olives) (Hurtado et al., 2012). The exact and complete definition of all trade and commercial preparations is described in the "Trade Standard Applying to Table Olives" (IOOC, 2004). The two main commercial productions of table olives, caustic-treated olives (Spanish) and olives dipped in brine (Greek style), are industrially carried out. However, these processes are difficult to monitor and control. The quality and safety standards of the final products cannot be guaranteed (Tufariello et al., 2016). NaOH used in the Spanish method is a simple and easy application. However, its debittering effect simultaneously causes the removal or disintegrating of many aroma components with molecules important for nutrition and health. In addition, the process generates a high volume of heavily contaminated wastewater. For these reasons, bitterness removal of olives with NaOH is considered risky in terms of environmental pollution (García et al., 2008).

Salt intake, used at high rates in table olives, is an important issue as it is associated with hypertension and cardiovascular diseases (Organization, 2012). It is an important problem, especially in countries with high daily NaCl intake. Therefore, the formulation development of low-salt foods is one of the most important health issues for consumers (Fadda et al., 2014).

Consumers' interest in natural and healthy foods is increasing. Accordingly, manufacturers demand the development of new techniques. Therefore, new techniques are being developed in table olive production to reduce potential spoilage from farm to fork, reduce microbial load, improve fermentation kinetics and ensure the safety of packaged products (Campus et al., 2018).

Ultrasound is sound waves produced at frequencies that are too high for the human ear to perceive. (Habibi et al., 2015). Using ultrasound in the debittering process of olive fruits is a viable technique that shortens processing time and reduces the NaOH concentration, resulting in a significant reduction in water usage and the number of washing cycles required to complete the debittering (Campus et al., 2018).

It was aimed to develop a new process in table olive production using different temperatures and NaOH concentrations. Ultrasound increased the NaOH penetration rate; therefore, higher oleuropein hydrolysis occurred. The processing time was reduced by 48% compared to the traditional method. More studies are needed to examine the effectiveness of ultrasound in table olive production.

The production of table olives by partial drying is a new practice to obtain products with low salt content in a short time without using chemicals. After different pretreatment applications, black table olives were produced by partial drying in the microwave. This way, it is aimed to produce salt-free or low-salt black table olives at a low cost. This production method has superior characteristics compared to the methods commonly used in table olive production.

2. MATERIALS AND METHODS

Materials

In the study, olives of the "Gemlik" variety harvested from Bursa-Gemlik (Türkiye) district were used. Before processing the olives, unsuitable crushed and rotten olives were removed, and homogenous olives were used in the experiment.

Methods

Debittering of Black Olives in Belt Microwave Dryer

Three pretreatments and control treatments without any pretreatment were performed before the microwave drying of olives. Pretreatment is given in Table 1.

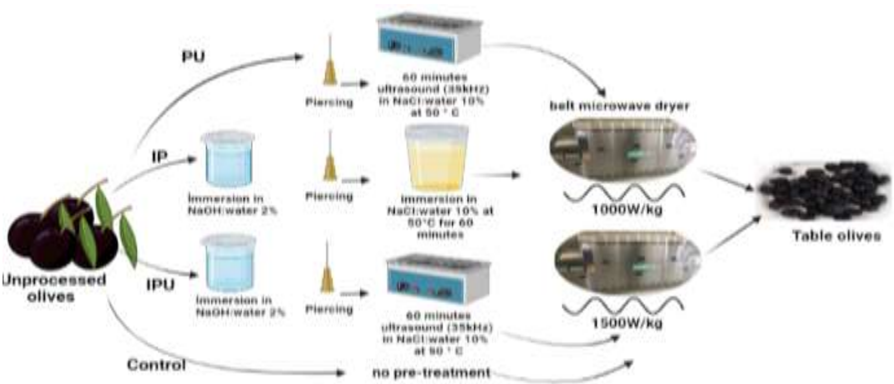


Figure 1. Debittering process of black olives in microwave dryer with different pretreatment applications pretreatments; PU: Piercing + Ultrasound, IP: Immersion In 2% NaOH + Piercing + Immersion In 10% Brine, IPU: Immersion In 2% NaOH + Piercing + Ultrasound.

Pilot scale belt microwave dryer, which a specially designed, was used to dry the olives. The microwave drying system has 8 magnetrons, each with 1000W power, and the belt length is 2 m. Using four magnetrons in the cross position, different microwave power was applicated by adjusting 1000W on / off: 20 s/60s and 1500 W by on/off 37s/60s. The band velocity of the microwave was adjusted to be 0.28 m / min, and the internal air suction was 1200 m^3 / h. The sensory bitterness value was determined by panelists and taking samples at 10minute intervals during drying. Drying was terminated when the sensory bitterness value was below 1.5. The physicochemical and sensory properties of olives produced after drying were determined. All treatments were done in triplicate.

Physical Analyses

Moisture contents were determined with TS 1129-ISO 1026 official method. In this method, samples were weighed on 2 g aluminum cups and kept under a vacuum at 70◦C for 6 hours (Institue, 1998). Hunter Lab D25-PC2 model colorimeter (Hunter Lab, Reston, USA) device was used for the color analysis of olive samples. The pH of the samples was measured by Mettler Toledo (Switzerland). The salt content of the olive samples was determined using the Mohr method (Cemeroğlu, 2013). The water activities of the dried olives, whose bitterness was removed, were carried out using a water activity measuring device (Novasina AG, Switzerland). Olive texture hardness was measured using a computer-integrated texture analyzer (Shimatsu, Japan) with a conical probe had 3 mm diameter and 2 mm cone length. Texture hardness (Newton) was determined as the maximum resistance force of olive tissue (the peak of resistance graphic). To determine the total titratable acidity, olive samples were homogenized in a blender. 20 g of the homogenized samples were weighed, and 100 ml of distilled water was added, mixed for 2-5 minutes, and made up to 250 ml with distilled water. Then the samples were titrated with 0.1 N NaOH until the pH value decreased to 8.1 and calculated and expressed as total titratable oleic acid (Cemeroğlu, 2013). All samples were analyzed in triplicate.

Brine Process

To produce black olives using the conventional method, olives were harvested when the black colour covered more than half of the olive flesh. After sorting, washing, and grading, the olives were placed in 10% salt brine. The olives in the brine were left to ferment by applying a pressure of 3.5 kg/m^2 (Özdemir, 2011).

Sensory Analysis of Bitterness

Sensory analysis of olives was conducted with 10 panelists consisting of food and agricultural engineers. Sensory analyses performed during storage were performed quarterly for 15 months. Panelists were selected from non-smokers who had previously participated in sensory tests as panelists. The panelists evaluated the appearance, color, odor, texture, ease of separation from the pit, flavor, and general eating quality of processed olives (Panagou et al., 2002). In the sensory analysis to determine the decrease in bitterness of olives during partial drying in a microwave dryer, the "bitterness value" was determined by the scoring (0-9) method by 5 panelists.

Microbiological Analysis

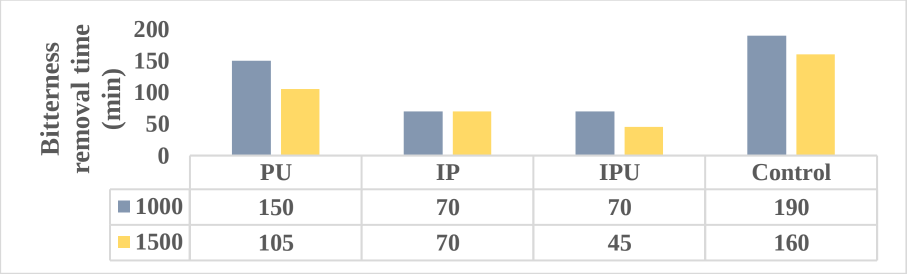
Microbiological analysis was done according to the official method (FDA, 2001). Mold and yeast counts were done in DRBC agar medium, 0.1 ml was sown on the surface of the medium by smear method. It was incubated at 25±1^{o}C for 5 days. Total viable counts were done in PCA medium by incubation at 35±1°C for 48 hours.

Statistical Analysis

The data in the study were shown as mean value ± standard deviation. Statgraphics software was used for the statistical analyses. Differences were considered significant when $p < 0.05$. Tukey test was used for the multiple comparisons (Kayahan ve Saloğlu, 2021).

3. RESULTS AND DISCUSSION

Debittering times required for olives with two different microwave power and four different pretreatment applications were determined in this study. Different pretreatment applications were used to accelerate the olive bitterness removal process. The effects of different pretreatments and microwave powers on debittering time of olives were given in Figure 2.



	PU	IP	IPU	Control
1000	150	70	70	190
1500	105	70	45	160

Figure 2. Olive bitterness removal time in microwave drying

Different pretreatments and microwave power were found to affect the debittering time of olives between 45-190 minutes. The bitterness of IPU applied olives was removed in 45 minutes in a microwave dryer with 1500 W power. Thus, these olives were determined as the group whose bitterness was removed in the shortest time. The longest olive debittering time was determined in the control group of olives with 1000 W microwave power. While the longest debittering time was observed in the control group, especially at both microwave powers, it was determined that all pretreatment applications, especially IPU application, reduced the debittering time to less than half a minute. As the microwave power increased, the debittering time decreased, similarly in a study where the black olive slices were dried using a microwave cabinet at three different power levels (180, 360 and 540 W). It was reported that the drying time of the olive slices decreased significantly as the microwave power increased (İçier et al., 2014).

The effect of different applications on the color of table olives in the microwave debittering process was statistically investigated. The color values of microwave debittered, brine processed and raw olives in brine were given in Table 1.

When the L, a, b color values of olives were compared with the process of removing bitterness using the raw olive and brine process, the L value of raw olives was 33.80, a value was 1.22, b value was 0.48; L value was 31.47, a value 3.53, b value 0.97 in brined olives. The highest L value in the microwave was observed to be brighter and similar to the brine process in the IPU application. For a and b values, the highest IP application was detected. It was observed that the pretreatment of microwave debittered olives significantly affected olive color values. When the methods used in convection olive production were compared, results close to the color values of the products obtained from the olives using IP and IPU applications were obtained.

On the other hand, İçier et al. (2014) determined that temperature increase and weight loss were the highest during the drying of olive slices at 540 W microwave power. The change in color values (L, a, b) of black olive slices for each microwave power level (180, 360 and 540 W) during drying was statistically significant. The greatest brightness change occurred in drying at 360 W power. It has been reported that bright color and high tissue hardness are the main criteria that table olives should have (Castro-Garcia et al., 2009; Kumral et al., 2009). In a study in which the bitterness of olives belonging to 14 different Italian cultivars was removed in an airflow dryer, it was stated that the bitterness of olives with pre-drilling was removed in the shortest time, but the sensory analysis results were highest in olives in which salting was applied (Piga et al., 2001).

Table 1. Color analysis results of olives debittered in microwave

	Raw Olives	Brine process	Power	Control	PU	IP	IPU	Avg	CV
L	33,80±1,53	31,47±0,1	1000	30,77±0,55	29,66±0,84	29,49±0,69	32,42±0,88	30,59	2,92
			1500	31,07±0,34	29,34±0,93	30,75±0,87	30,71±2,68	30,47	
			Avg	30,92AB	29,5C	30,12BC	31,56A		
a	1,22±0,7	3,53±0,03	1000	2,53±0,14b	1,93±0,09d	3,11±0,15a	2,13±0,13cd	2,43A	9,8
			1500	0,95±0,21e	1,24±0,25e	2,29±0,15bc	2,20±0,07bcd	1,67B	
			Avg	1,74C	1,59C	2,70A	2,16B		
b	0,48±0,5	0,97±0,2	1000	0,72±0,02	0,83±0,13	1,16±0,28	0,92±0,16	0,91	9,76
			1500	0,71±0,13	0,56±0,09	1,18±0,02	0,80±0,05		
			Avg	0,71B	0,69B	1,17A	2,34±0,13bc	0,81	

The physical analysis results of processed and raw olives were given in Table 2. The highest hardness was observed in the PU and control groups among the olives whose bitterness was removed by microwave drying. In the traditional method, the hardness was determined as 2.20 N, and the lowest hardness value of 1.86 N was obtained in the drying application made with the IP method. It has been determined that the pH values of the samples were very close to each other, and the IPU sample has a higher pH value. The pH values of olives produced by the brine process were found to be 4.59 due to lactic acid fermentation.

Table 2. Physical analysis results of processed and raw olives

	Raw olives	Brine process	Power	Control	PU	IP	IPU	Avg	CV
Hardness (N)	2,06±0,13	2,20±0,10	1000	2,19b±0,24	3,40a±0,07	1,46c±0,41	2,49b±0,52	2,38	8
			1500	2,62b±0,19	2,25b±0,29	2,27b±0,38	2,23b±0,05	2,34	
			Avg	**2,40AB**	**2,82A**	1,86C	2,36B		
pH	6,30±0,06	4,59±0,02	1000	6,24±0,055	6,17±0,015	6,16±0,035	6,34±0,01	**6,22A**	0,73
			1500	6,13±0,01	6,19±0,02	6,11±0,035	6,22±0,055	6,16B	
			Avg	6,18B	6,18B	6,13B	**6,28A**		
Titr. acid (%Oleic acid)	1,73±0,01	0,72±0,01	1000	2,2±0,03c	2,045±0,07d	1,58±0,02e	1,535±0,01e	1,84B	2,39
			1500	**2,8±0,01a**	2,43±0,05b	2,005±0,05d	2,12±0,01cd	2,34B	
			Avg	2,50A	2,24B	1,79C	1,83C		
Dry Matter (%)	49,95±1,23	60,57±1,35	1000	64,35±3,05	60,96±1,72	55,92±3,2	58,58±0,91	59,95B	4,41
			1500	80,12±2,8	72,01±0,7	60,91±0,41	62,39±1,79	**68,86A**	
			Avg	**72,23A**	66,48B	58,41C	60,49C		
Water activity	0,93±0,01	0,86±0,02	1000	0,91±0,01	0,90±0,007	0,91±0,02	0,92±0,002	0,91	1,05
			1500	0,88±0,003	0,90±0,005	0,92±0,05	0,91±0,07	0,90	
			Avg	0,90B	0,90B	**0,92A**	**0,91A**		
Salt (%)			1000	-	-	0,22±0,05	0,31±0,1	0,27	3,16
			1500	-	-	0,17±0,06	0,26±0,08	0,22	
			Avg	-	-	0,20B	0,29A		

The highest dry matter content was determined in control samples in both microwave power values. As expected, drying powers were effective on dry matter content. In addition, it was determined higher in the brine or control. Statistically, lower water activity was determined in the samples with the high dry matter. The water activity of control and PU groups was found to be lower than the other groups. While the salt content could not be determined in control and PU groups, it was determined in the range of 0.17-0.31% in the IP and IPU groups.

Products with high sensory values could easily find a place on the market shelves. For this reason, it is important to determine physical and sensory properties. Sensory scores of processed were given in Table 3.

Table 3. Sensory analysis results of processed olives

	Brine process	**Power**	**Control**	**PU**	**IP**	**IPU**	**Average**	**CV**
Color	6,47 ± 3,52	1000	5,33±0,95	5,83±0,74	5,33±0,81	5,5±1,15	5,50	8
		1500	5,5±0,94	5,67±0,69	5±0,74	5,33±1,10	5,38	
		Avg	5,42	5,75	5,17	5,42		
Smell	6,67 ± 1,43	1000	4,83±0,68	5±1,06	4,5±1,34	4,83±1,06	4,79	6,07
		1500	5,17±0,68	4,83±1	4,17±1,38	5,17±1,06	4,83	
		Avg	5	4,92	5	4,33		
Taste	6,09 ± 1,22	1000	4,00±0,89	5,50±1,15	2,67±1,11	4,33±1,73	4,13	7,64
		1500	3,17±0,81	5,00±1,7	3,50±1,37	5,00±1,10	4,17	
		Avg	3,58C	**5,25A**	3,08C	**4,67AB**		
Tissue	6,51 ± 1,32	1000	3,5±1,06	5±1,15	3,5±1,1	4,5±0,68	4,13	6,2
		1500	3,33±1,24	4±0,81	3,33±1,38	4,83±0,96	3,87	
		Avg	3,42B	**4,5A**	3,42B	**4,67A**		
Shell separation	5,93 ± 1,80	1000	4,33±0,30	4,52±0,78	4,03±0,75	4,50±0,89	4,32	7,77
		1500	3,67±0,34	3,67±0,86	3,67±0,88	4,33±0,64	3,83	
		Avg	3,75	3,83	3,58	4,17		
Seed separation	5,92 ± 1,20	1000	5,83±1,24	5,67±1,69	5,33±1,24	5,17±0,94	5,5	4,92
		1500	5,67±1,21	6±1,41	5±0,95	5,83±0,81	5,63	
		Avg	5,75	5,83	5,17	5,5		
General view	5,87 ± 1,35	1000	3,33±0,68	5,33±0,47	3,83±0,94	4,16±1,34	4,17	6,97
		1500	3±0,81	4,67±1,29	3,83±0,89	4,66±0,89	4,04	
		Avg	3,17C	**5,00A**	3,83BC	**4,42AB**		
Sensory analysis average	6,26 ± 1,21	1000	4,35±0,39	5,19±0,75	4,09±0,69	4,61±0,8	4,57	3,56
		1500	4,19±0,32	4,83±0,84	4,14±0,89	4,97±0,55	4,54	
		Avg	4,27BC	**5,01A**	4,12C	**4,80AB**		

No statistically significant difference was determined in the sensory analysis of color, odor, skin and seed separation properties. The fact that the color of the olives was black and their brightness was similar to each other was thought to be effective in this result. Similar values may have been determined in the odor evaluation since an odor that could be defined as a defect was not identified. Skin separation has been reported as a defect that could be found in olives after debittering with the hot air drying process (Özdemir et al., 2015). However, it has been determined that this criterion is not at the level that can be defined as a defect in the debittering experiments with microwave drying, and it may be due to the close values of the samples. Olive variety was found to be more effective than the pretreatment and drying and debittering processes for seed separation feature of table olive. Therefore the seed separation feature did not differ among the samples.

One of the most important criteria in sensory properties is taste. When the taste of the microwaved olives was examined, the highest PU and IPU pretreatments were appreciated. Likewise, in general appearance and sensory analysis, it was found that statistically, the applications where PU and IPU pre-process were performed came to the fore. It was observed that the sensory score average of Gemlik olives processed with the natural brine method was higher than the olives obtained by microwave drying. This result is because the sensory properties of fermented olives are more usual. In Türkiye, almost all Gemlik olives are processed using natural brine.

It has been reported that flavor and texture properties improve (Piga et al., 2001) and the color becomes more attractive to consumers due to the interaction of pigments and phenol oxidation with fermentation (Poiana ve Romeo, 2006). In this study, there is no fermentation process in the pretreatment and microwave debittering experiments. For this reason, it is thought that aromatic components resulting from fermentation cannot occur. However, aromatic components can be formed in the partial drying process to reduce bitterness in the microwave. Piga et al. (2001) and Poiana and Romeo

(2006) observed that features of olives (texture hardness, sensory analysis values and color analysis values) were compatible with the literature and had marketable value. According to the sensory and physical analysis in this study, PU pretreatment and 1000W microwave power parameters were chosen as the best method. In the second stage of the study, shelf stability of the selected method for table olive bitterness removal was investigated. Table black olives were produced with the selected method, and different pasteurization methods were examined for 15 months, mold and yeast count, and total viable count (Figure 3).

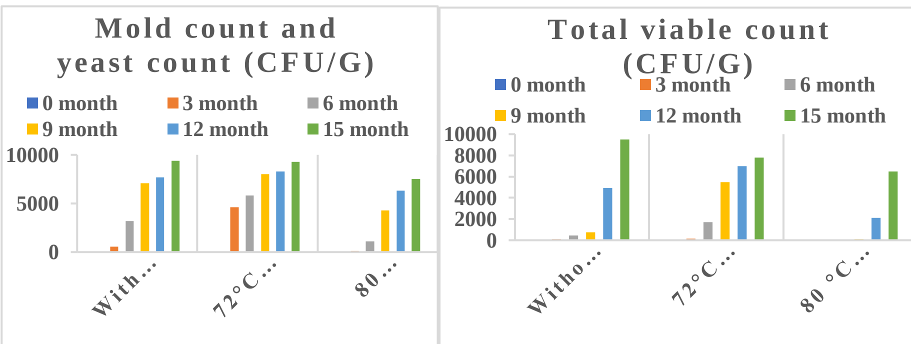


Figure 3. Mold count and yeast count and total viable count results during shelf life of microwaved debittered olives

Mould, yeast, and total viable counts were made every 3 months for 15 months. Although the olives did not contain salt, olives were appropriate for the Turkish Food Codex Microbiological Criteria Communique, limiting the mold count 10000 cfu/g. It is thought that this is caused by the debittering and packaging method used in the study. The water activity of olives was lowered (0.79-0.86) due to the removal of bitterness by drying and the use of a multi-effect system in packaging. Mixing apple vinegar to olives provides optimum acidity, making them suitable for packaging and pasteurization, can be conserved at 4°C for 15 months.

4. CONCLUSION

In this study, different pretreatment and microwave power of table olive production with partial drying in the microwave were investigated. One of the most important cost items in producing naturally dried olives is the energy spent on reducing bitterness, and debittering with a microwave dryer could greatly reduce the cost. In addition, it has been observed that debittering with microwave drying has similar sensory and chemical properties to natural brine black olive production methods.

When all the analyzed parameters were evaluated in general, it was seen that IPU and PU pretreatments and debittering in a 1500 W microwave dryer were recommended for naturally dried olive production. It is thought that the new methods that will produce natural, nutritious, and low-salt olives with low costs will provide competitive power to the producers. This study is expected to be able to contribute to the producers in this respect.

It has been reported that flavor and texture properties improve, and the color becomes more attractive to consumers due to the interaction of different pigments and phenol oxidation with fermentation. In this study, there is no fermentation process in the pretreatment and microwave debittering experiments. For this reason, it was thought that aromatic components resulting from fermentation could not occur. However, aromatic components can form during the partial drying process to remove bitterness in the microwave. This partial drying process helps decrease bitterness and increase the taste components of olives.

Acknowledgment

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REFERENCES

Campus, M., Değirmencioğlu, N., Comunian, R. (2018). Technologies and trends to improve table olive quality and safety. *Frontiers in Microbiology* 9(617):23.

Castro-Garcia, S., Rosa, U. A., Gliever, C. J., Smith, D., Burns, J. K., Krueger, W. H., Ferguson L., Glozer, K. (2009). Video evaluation of table olive damage during harvest with a canopy shaker. *American Society for Horticultural Science* 19(2): 260-266.

Cemeroğlu, B. (2013). *Gıda Analizleri*. Ankara: Bizim Grup Basımevi.

Fadda, C., Del Caro, A., Sanguinetti, A. M., Piga, A. (2014). Texture and antioxidant evolution of naturally green table olives as affected by different sodium chloride brine concentrations. *Grasas Aceites 65*(1):1-141.

FDA. (2001). Bacteriological Analytical Manual In *Aerobic Plate Count* (Vol. 3).

García, A., Romero, C., Medina, E., García, P., De Castro, A., Brenes, M. (2008). Debittering of olives by polyphenol oxidation. *Journal of Agricultural Food Chemistry 56*(24):11862-11867.

Habibi, M., Golmakani, M.T., Mesbahi, G., Majzoobi, M., Farahnaky, A. (2015). Ultrasound-accelerated debittering of olive fruits. *Innovative Food Science Emerging Technologies 31*:105-115.

Hurtado, A., Reguant, C., Bordons, A., Rozès, N. (2012). Lactic acid bacteria from fermented table olives. *Food Microbiology 31*(1):1-8.

İçier, F., Baysal, T., Taştan, Ö., Özkan, G. (2014). Microwave drying of black olive slices: effects on total phenolic contents and colour. *Gıda 39*(6):323-330.

Institue, T.S. (1998). Fruit and vegetable products–determination of dry matter by drying under low pressure and water content by azeotropic distillation. In (Vol. TS 1129 ISO 1026). Ankara.

IOOC. (2004). International Olive Oil Council In *Trade Standard Applying To Table Olives* (Vol. COI/T20/Doc No 1.). Madrid.

Kayahan, S., Saloglu, D. (2021). Comparison of phenolic compounds and antioxidant activities of raw and cooked Turkish Artichoke cultivars. *Frontiers in Sustainable Food Systems 5*:8.

Kumral, A., Basoglu, F., Sahin, I. (2009). Effect of the use of different lactic starters on the microbiological and physicochemical characteristics of naturally black table olives of Gemlik cultivar. *Journal Of Food Processing Preservation 33*(5):651-664.

Organization, W.H. (2012). Good Health adds life to years:. In *Global brief for World Health Day 2012*: World Health Organization.

Özdemir, Y. (2011). *Bazı melez zeytinlerin fizikokimyasal özelliklerinin ve starter kültür (Lactobacillus Plantarum) ilaveli sofralık zeytin fermentasyonuna uygunluklarının belirlenmesi* (Doktora Tezi), Namık Kemal Üniversitesi, Fen Bilimleri Enstitüsü, Tekirdağ.

Özdemir, Y., Güven, E., Öztürk, A. (2015). Debittering of olives by semi drying. *Pamukkale Üniversitesi Mühendislik Bilimleri Dergisi 21*(9):390-393.

Panagou, E., Tassou, C., Katsaboxakis, K. (2002). Microbiological, physicochemical and organoleptic changes in dry-salted olives of thassos variety stored under different modified atmospheres at 4 and 20° C. *International Journal Of Food Science Technology 37*(6):635-641.

Perpetuini, G., Prete, R., Garcia-Gonzalez, N., Khairul Alam, M., Corsetti, A. (2020). Table Olives more than a fermented food. *Foods 9*(2):178.

Piga, A., Gambella, F., Vacca, V., Agabbio, M.C.S. (2001). Response of three sardinian olive cultivars to greek-style processing. *Italian Journal of Food Science 13*(1):29-40.

Poiana, M., Romeo, F.V. (2006). Changes in chemical and microbiological parameters of some varieties of sicily olives during natural fermentation. *Grasas Y Aceites 57*(4):402-408.

CHAPTER 4

THE EFFECTS OF HUMIC ACID AND SEAWEED APPLICATIONS ON GERMINATION AND SEEDLING DEVELOPMENT IN SPINACH (*Spinacia oleracea L.*)

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1. INTRODUCTION

The spinach plant grows during a short vegetation period. Therefore, fertilization is important in spinach cultivation to obtain high-quality products and high yields per unit area (Yılmaz et al., 2012). In addition to hybrid cultivars resistant or tolerant to adverse environmental conditions, practices that accelerate the initial development cycles of plants and ensure better root development and above-ground organs have become of great importance in recent years. In particular, it was determined that humic acid, one of the groups of organic substances, increases plant biomass, and this positive effect is greater in root development (Kaya et al., 2005). The researchers stated that humic acid helps uptake nutrients by increasing the permeability of the cell membrane in plants and reported that it has a positive effect on plant development due to the hormone-like substances in their structure (Demir et al., 2011). Humic substances are known as stimulants of seed germination and plant growth, and they can pass through the plant membranes, facilitating the transport of trace elements in the plant roots (Aşık et al., 2012).

In the studies of Özenç and Şenlikoğlu (2017), the spinach plants grown in soils where compost was added and nitrogen fertilizer was applied in greenhouse conditions during the development were investigated. Nitrogen, hazelnut husk compost, manure and enriched compost were applied in the experiment. When all the data were evaluated, it was found that mixing 8% enriched compost with nitrogen fertilizer applied to the soil increased plant development. It has been recommended to use compost products regularly as it supports fertilizer application.

Gülser and Ayaş (2016) used four doses of sulfur (0, 125, 250, 375 g HA m^{-2}) and humic acid (0, 10, 20, 30 g HA m^{-2}) in their study on spinach. In addition, urea (40 kg N da^{-1}), TSP (30 kg P_2O_5 da^{-1}) and manure (3.5 tons da^{-1}) were applied to all parcels to promote plant development. It was determined that the effect of sulfur applications on Fe, Cu and Zn contents of spinach was significantly higher ($p<0.05$), increasing the Cu and Zn content. By applying humic acid, significant increases in the Fe, Zn and Mn contents of spinach have been achieved ($p<0.01$).

Akyurt et al. (2011) applied liquid organic fertilizer obtained from *Ulva lactuca* (*Chlorophyta*) plant in their study. Chemical fertilizer (20:20:20) was also used. In experiments to determine the effects of fertilizers on germination, broccoli (*Brassica oleracea* L.) and spinach (*Spinacia oleracea* L.) seeds were used. Twenty-five seeds were sown in each replication. Liquid organic and suspension chemical fertilizers were applied to the soil before sowing. The average germination time is 10 days. The germination rate (%) of broccoli seeds was found to be 45% in the control group, 50% in the artificial fertilizer group, and 85% in the liquid organic fertilizer group. In spinach plants, germination rates were determined as 72% in the control group, 62% in artificial fertilizer and 70% in the liquid organic fertilizer group. Liquid organic fertilizer increased the germination rate of broccoli seeds by 40%, while it was ineffective in germinating spinach seeds.

Barley (1961) reported that vermicompost contains 5 times the amount of N in usable form in the soil, 7 times the amount of K, and 3 times the amount of Ca. Senesi et al. (1990) stated that when humic acid is applied to soil or nutrient media has a positive effect on the dry weight of peas, the nutrient uptake, and the seed germination.

It has been reported that the application of humic compounds promotes seed germination in various plant species by increasing the enzymatic activities in seed tissues during germination, and increasing the germination rate, root and shoot growth (Rauthan and Schnitzer, 1981). One of the most important properties of humic acid is that it is a biological solvent for plants thanks to the leonardite it contains (Güneş, 2007).

In corn (Canellas et al., 2009), pepper (Cimrin et al., 2010), and wheat (Tahir et al., 2011), it has been determined that humic acid applications have a positive effect on seedling root development.

Kolsarıcı et al. (2005) determined the effect of 60 gr humic acid application on sunflower (*Helianthus annuus* L.) seedling development. As a result of the study, it was found that humic acid increases root length, plant height and seedling dry weight.

Dursun et al. (1999) reported that 50, 100, 150 and 200 ml/l doses of humic acid application to tomato and eggplant seedlings increased the leaf number, macro and micronutrient content, leaf area, fresh and dry weights of roots and stems. It was also found that 0, 640, 1280 and 2560 mg/l concentrations of humic acid application on tomato seedlings in greenhouse positively affect growth and nutrient uptake (David et al., 1994; Güngör, 2018).

Erdal et al. (2000) investigated the effect of humic acid and phosphorus application at different concentrations to a calcareous soil on corn development (*Zea mays* L.). For this purpose, 4 doses of phosphorus (0, 20, 40, 80 mg/kg) and 3 doses of humic acid (0, 250, 500 mg/kg) were applied to the soil. As a result, it was found that humic acid applications increased the concentration, P uptake, and plant dry weight.

Tüzel et al. (2011), in a study conducted on organic lettuce-salad cultivation, the highest yield among biofarm, humic acid and leonardite applications was obtained by Biofarm + humic acid application in the first year and biofarm application in the second year. Gülser et al. (2014) investigated the effect of humic acid, salicylic acid and potassium applications on tomatoes. The highest germination rate was found in the humic acid application at 80%.

When seaweeds are mixed directly into the soil, it is aimed to improve soil structure and maintain soil fertility (Güner ve Aysel, 1996). By providing strong root development, they enable plants to take more nutrients and water from the soil, increase vegetative development by accelerating the chlorophyll formation and increase the resistance to plant diseases, pests and environmental stress. They increase yield and quality, and long-term intake of macro and micronutrients from the soil (Blunden et al., 1992; Hong et al., 1995; Özenç ve Şen, 2017).

Özenç and Şen (2017) investigated the effects on plant development and some quality characteristics of grafted and ungrafted tomato plants grown in soils applied seaweed fertilizer at different development periods in the greenhouse. Application of liquid seaweed fertilizer to the soil supported plant development and increased nutrient element content in both tomato cultivars. The best development was achieved in the applications carried out during the seedling period of the grafted cultivar. In this cultivar, 2 doses of fertilizer were applied to the soil during the seedling period, increased plant height (177.78 cm), yield (5919 g) and fruit number (105 fruit).

In biological agriculture and horticulture, diluted extracts of seaweed are applied to promote growth, prevent pests and diseases and improve the fruit quality. The efficacy of the extracts is probably based upon plant hormones (mainly cytokinins) and trace nutrients present in the extracts. It was found that root growth was stimulated in cucumber grown by seaweed extract once a week under greenhouse conditions, and the total dry weight increased by 50% (Verkleij, 1992). According to Fan et al. (2013), brown seaweed extracts increased the total soluble protein content, antioxidant capacity, phenolic and flavonoid contents of spinach.

Demirkaya (2010) investigated the possibilities of using seaweed extract in osmotic conditioning applications performed on pepper (*Capsicum annuum* L.) and onion (*Allium cepa* L.) seeds. Osmotic conditioning treatments were conducted with the 1:500 seaweed extract solution at 20°C and 15°C in seeds of pepper and onion, respectively, for 1, 2 and 3 days. Osmotic conditioning treatments with seaweed extract increased germination rate and reduced mean germination time in pepper and onion seeds. The shortest mean germination time of pepper seeds was 5.6 days in the 2-day application of cv. Demre Sivri, 8.3 days in the 2-day application of cv. Kandil Dolma and 6.5 days in the 2-day application of cv. Yalova Çarliston while the mean germination times of control seeds were 7.2, 9.7 and 7.6 days, respectively. The shortest mean germination time of onion seeds was 3.5 days in the 3-day application of cv. TEG-502 and 3.2 days in the 3-day application of cv. Contes, while the mean germination times of control seeds were 5.4 and 5.5 days, respectively.

Demirkaya (2012) investigated the possibilities of using seaweed extract (Maxicrop) in tomato seeds (*Lycopersicum esculentum*) in Rio Grande, H-2274 and SCI-21 cultivars. Osmotic conditioning treatments were conducted with the 1:500 seaweed extract solution at 20°C in seeds of tomato for 1, 2, and 3 days. Osmotic conditioning treatments with seaweed extract increased germination and emergence rates and shortened the mean germination time and mean emergence time of the seeds of the three tomato cultivars. The highest emergence ratio of seeds was 90% in cv. Rio Grande, 87.12% in cv. H-2274 and 62% in cv. SCI-21 in the 3-day applications, while emergence ratios of control seeds were 82%, 76.6% and 46%, respectively.

Anjos Neto et al. (2020) evaluated the effects of seed priming with SWE (Ascophyllum nodosum) on the germination, seedling growth and antioxidant capacity of spinach seedlings under conditions of heat stress. Five concentrations of SWE (0.0, 0.15, 0.30, 0.60 and 1.2%) were used for priming the seeds under two temperatures (15 and 30°C). The study showed that seed priming with SWE was effective in mitigating stress due to high temperatures and improving spinach seed germination and seedling vigor.

Sasikala et al. (2016) observed the efficiency of the Seaweed Liquid Extract by performing the experiments at different concentrations such as 0.2%, 0.4%, 0.6%, 0.8% and 1%. Seaweed extracts act as biostimulants mainly due to the presence of plant hormones. The phytohormones identified in seaweed extracts are auxins, cytokinins, gibberellins, abscisic acid and ethylene. The objective of the study was to increase the soil fertility using algal extract (*Sargassum tenerrimum*) as a fertilizer and also to improve the seed germination, growth, yield as well as quality for better production. According to the results, it was clear that maximum growth and yield of the tomato plant can be achieved at 0.6% concentration. Even though leaf number, leaf area, and root length were high at 0.8 % it did not deviate much from 0.6%. It has been emphasized that higher concentrations may be inhibited the growth and yield of the tomato plant.

2. MATERIAL AND METHODS

Material

In the experiment, “Matador” spinach (*Spinacia oleracea*) cultivar was used as the plant material, peat and perlite were used as the medium, and seaweed and humic acid were used as fertilizer.

Matador is a large-leaved spinach cultivar. The leaves are plump, short-stemmed, dark green, smooth, and the leaf tips are oval. It is a productive and rapid growing cultivar. It appears broad on the soil. In all regions, sowing is carried out. It is resistant to cold and transportation. A temperature of 16-25 degrees is the optimal temperature for germination and development (Anonymous, 2022).

Climatic characteristics of the experimental greenhouse

The average temperature in the greenhouse during the experimental period is suitable for spinach cultivation. Spinach is a cool climate vegetable. Therefore, it does not need heating undercover in the Mediterranean Region. The germination time is between 16-20^o in the daytime and 5-8^o at night. Average humidity values are also suitable.

Methods

The study was conducted in the experimental greenhouse of Cukurova University Kozan Vocational School between December 17, 2020 and January 28, 2021. In the study, the rooting status of Matador cultivar seeds was determined by applying fertilizers of various organic origins. The applications in the experiment are given in Table 1. Spinach seeds were sowed in viols with 30 seeds for each application. Fertilizers were also applied when sowing seeds to perlite and peat media. The same amount of water was given to each application, including the control group.

Preparation of the Fertilizers

In the experiment, 500 ppm doses of humic acid and 750 ppm doses of seaweed were used. These doses were applied to the soil before sowing seeds on 15.12.2020.

Table 1. Applications used in the experiment

Applications	Doses
Peat	There is no application of any fertilizer
Peat + Humic acid	500 ppm
Peat + Seaweed	750 ppm
Perlite	There is no application of any fertilizer
Perlite + Humic acid	500 ppm
Perlite + Seaweed	750 ppm

Germination Status

The viols in the greenhouse were checked every day to see if there was any germination. When the seeds burgeon by 2 mm, they have been evaluated as germinated. Sowings were made on December 15, 2020. Humic acid and seaweed were given to the progress media when the first sowings were made. Every day it was checked whether there was germination in the seeds. From the sowing of the seeds until the root evaluations were made, the temperature in the greenhouse was around 16-20 degrees during the day and 5-8 degrees at night. The placement of viols at the greenhouse has been made to receive light sun. Irrigation was carried out once a week by adding nutrients to the irrigation water. In total, irrigation was carried out 5 times with seaweed and humic acid, and intermediate irrigations were carried out with ordinary irrigation water.

Figure 1. An image of the greenhouse the experiment was conducted

Figure 2. Images of spinach seeds sown on December 15, 2020 (Peat+Seaweed, Peat+Humic acid, Peat, Perlite+Seaweed, Perlite+Humic acid, Perlite)

3. RESULTS AND DISCUSSION

The first germination was on December 21, 2020, at the peat+seaweed application. On the same date, peat+humic acid, perlite+seaweed and perlite+humic acid and peat applications followed. No emergence has been seen in the perlite application where no any application was made. The germination period of seeds was determined as 7 days. On December 23, 2020, a few seed outflows were also observed in the perlite application. In all applications, some seeds did not germinate, and gaps were seen in places. This case has happened more in perlite than peat applications. On December 25, 2020, seed emergence was completed at viols of all the applications and reached the two-cotyledon stage. On January 2, 2021, the plant heights began to increase. On January 14, 2021, the seedlings have reached the size to be transplanted into the soil. The time from sowing the seeds to the full size of the seedlings was determined as 25-31 days. Plant growth has been monitored for more a while. Starting from January 28, 2021, the seedlings were removed and the root development was examined.

Kaya et al. (2005) reported that humic acid increased plant biomass and this positive effect is more in root development. Aşık et al. (2012) reported that humic substances as stimulants of plant germination and growth. Seaweeds enable plants to take more nutrients and water from the soil by providing strong root development, increasing green parts by accelerating chlorophyll formation in plants, provide resistance to diseases and pests, and environmental stresses (Blunden et al., 1992; Hong et al., 1995; Özenç ve Şen, 2017). It has been found that root growth is stimulated in cucumber plants grown by seaweed extract once a week in greenhouse conditions (Verkleij, 1992).

Gülser et al. (2014) found the highest germination rate of 80% in tomatoes with the humic acid application. Cimrin et al. (2010) determined that humic acid (HA) and phosphorus applications increased the growth and plant growth parameters. They concluded that high humic acid doses had positive effects on salt tolerance based on the plant growth parameters and nutrient contents.

Sasikala et al. (2016) obtained the maximum growth and yield of tomato plants at 0.6% concentration. Even though the leaf number, leaf area, and root length were high at 0.8 % it did not deviate much from 0.6%.

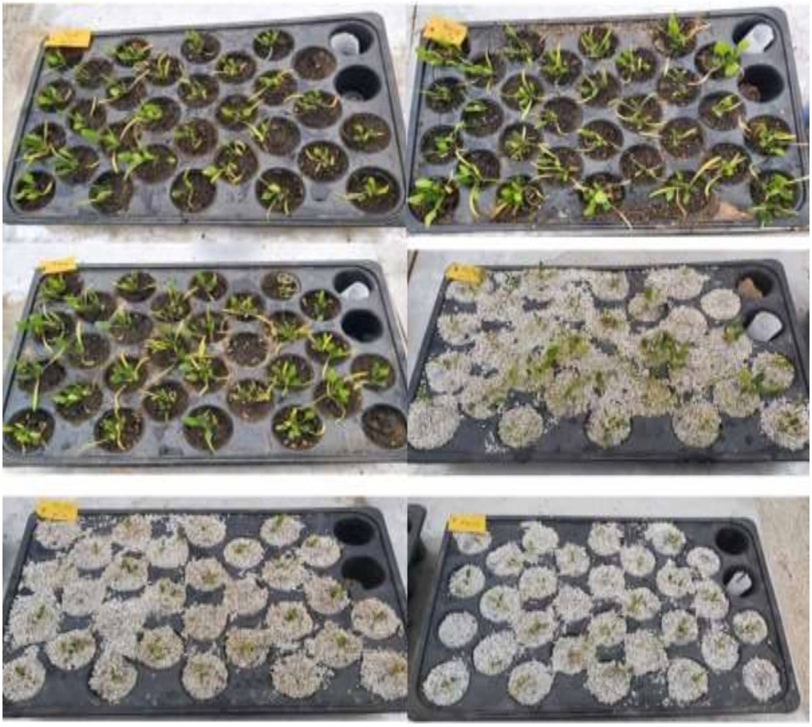

Figure 3. Development of spinach seedlings before uprooting on January 28, 2021 (Peat + Seaweed, Peat + Humic acid, Peat, Perlite + Seaweed, Perlite + Humic acid, Perlite)

Figure 4. Images of root development in spinach seedlings after transplanting (Peat + Seaweed, Peat, Peat + Humic acid, Perlite + Humic acid, Perlite + Seaweed, Perlite)

4. CONCLUSION

As a result of the study, it was determined that organic fertilizers such as humic acid and seaweed in spinach cultivation have positive effects on plant development, endurance, weight, especially early germination, and strong root development. Germination time was around 7 days in all fertilizer applied groups. The time from seed sowing to the completion of seedling development was determined as 25-31 days. It has been seen that especially peat+seaweed and peat+humic acid applications have better results than the other applications. It was thought that the fertilizers used in perlite media were less effective. Therefore, it is understood that peat and perlite media should be used together in germination studies. It is suggested that such organic fertilizer applications should be tested on different plant species and at different doses.

REFERENCES

Akyurt, İ., Şahin, Y., Koç, H. (2011). Deniz marulunun (*Ulva sp*.) sıvı organik gübre olarak değerlendirilmesi. *Karadeniz Fen Bilimleri Dergisi* 1(4): 55-62.

Anjos Neto, A.P., Oliveira, G.R.F., Costa Mello, S., Silva, M.S., Gomes-Junior, F.G., Luz Coelho Novembre, A.D., Azevedo, R.A. (2020). Seed priming with seaweed extract mitigate heat stress in spinach: effect on germination, seedling growth and antioxidant capacity. *Bragantia, Campinas*, 79(4):502-511.

Anonim (2022). http://www.sunagritohumculuk.com/urun/ispanak-matador#:~:text=Geni%C5%9F%20yaprak%C4%B1%20%C4%B1spanak%20%C3%A7e%C5%9Fididir.,%C3%A7e%C5%9Fittir.So%C4%9Fu%C4%9Fa%20ve%20nakliyeye%20dayan%C4%B1kl%C4%B1d%C4%B1r. Date of access: 11.03.2022.

Aşık, B.B., Turan M.A., Çelik H., Katkat, A.V. (2012). Yapraktan humik asit uygulamasının tuzlu ve kireçli toprak koşullarında buğday bitkisi gelişimi ve kimi besin elementi alımı üzerine etkisi. *Sakarya Üniversitesi Fen Edebiyat Dergisi.*

Barley, K.P. (1961). Plant nutrition levels of vermicast. *Advances in Agronomy*.13: 251.

Berger, K.C. (1949). Has compiled tables of the boron content and requirements of various *Crops. Avdan, Argon.*, 1: 321.

Blunden G., Whapham, C., Jenkins, T. (1992). Seaweed extracts in agriculture and horticulture: their origins, uses and modes of action. School of Pharmacy and Biomedical Science and "School of Biological Sciences, University of Portsmouth, King Henry John Street, Portsmouth, Hampshire P01 202, U.K.

Canellas, L.P., Spaccini, R., Piccolo, A. (2009). Relationships between chemical characteristics and root growth promotion of humic acids isolated from Brazilian oxisols. *Soil Science* 174: 611–620.

Cimrin, K., M., Önder, T., Turan, M., Burcu, T. (2010) Phosphorus and humic acid application alleviate salinity stress of pepper seedling. *African Journal of Biotechnology* 9:5845–5851.

David, P.P., Nelson, P.V., Sanders, D.C. (1994). A humic acid improves growth of tomato seedling in solution culture, *Journal of Plant Nutrition* (17) 173.

Demir, E., Çimrin, M.K. (2011). Arıtma çamuru ve hümik asit uygulamalarının mısırın gelişimi besin elementi ve ağır metal içerikleri ile bazı toprak özelliklerine etkisi. Ahi Evran Üniversitesi Ziraat Fakültesi Toprak Bilimi ve Bitki Besleme Bölümü, Kırşehir.

Demirkaya, M. (2010). Deniz yosunu (*Ascophyllum nodosum*) ekstraktı uygulamalarının biber ve soğan tohumlarının canlılığı ve gücüne etkileri. *Erciyes Üniversitesi Fen Bilimleri Enstitüsü Dergisi* 26(3):217-224.

Demirkaya, M. (2012). Deniz yosunu (*Ascophyllum nodosum*) ekstraktı uygulamalarının domates tohumlarının canlılığı ve gücü üzerine etkileri. *Alatarım*, 11 (1): 13-18.

Dursun, A., Güvenç, İ., Turan, M. (1999). Macro and micro nutrient contents of tomato and eggplant seedlings and their effects on seedling growth in relation to humic acid application. Improved Crop Quality by Nutrient Management, Anaç, D.; Martin-Prevel, P. Eds. Kluwer Academic Publishers, London 229.

Erdal, İ., Bozkurt, M.A., Çimrin, K.M., Karaca, S., Sağlam, M. (2000). Kireçli bir toprakta yetiştirilen mısır bitkisi (*Zea mays* L.) gelişimi ve fosfor alımı üzerine humik asit ve fosfor uygulamasının etkisi. *Turk J Agric For*, 24: 663-668.

Fan, D., Hodges, D., M., Critchley, A., T., Prithiviraj, B. (2013) A commercial extract of Brown Macroagla (*Ascophyllum nodosum*) affects yield and the nutritional quality of spinach in vitro. *Commun Soil Science Plant Anal* 44:1873–1884.

Gülser E., Tüfenkçi Ş. ve Demir, S. (2014). Domateste potasyum, salisilik asit ve humik asit uygulamalarının fide çıkışı ve fusarium solgunluğuna (*Fusarium oxysporum f.sp. lycopersici*) etkileri. *Yuzuncu Yil University Journal of Agricultural Sciences*, 24(1): 16-22.

Gülser, F., Ayaş, H.Ç. (2016). Kükürt ve humik asit uygulamalarının ıspanak (*Spinacea oleracea var. Spinoza*) bitkisinin mikro besin elementi içeriklerine etkisi. *Toprak Bilimi ve Bitki Besleme Dergisi* 4 (1): 27 -31.

Güner, H., Aysel, V. (1996). Tohumsuz Bitkiler Sistematiği. Ege Üniversitesi Fen Fakültesi Kitaplar Serisi, No.108. Bornova, İzmir.

Güneş, A. (2007). *Allüviyal materyaller üzerinde oluşan topraklarda yetiştirilen mısır bitkisinin (Zea mays L.) verim ve besin içeriği üzerine organik ve mineral gübre uygulamalarının etkisi*. (Yüksek lisans tezi) Atatürk Üniversitesi Fen Bilimleri Enstitüsü, Erzurum.

Güngör, K. (2018). *Hümik asit uygulamalarının mısır (Zea Mays L.) bitkisinin kök gelişimi ve besin elementleri alımına etkisi*. (Yüksek lisans tezi) Ankara Üniversitesi Fen Bilimleri Enstitüsü, Ankara.

Hong, Y.P., Chen, C.C., Cheng, H.L., Lyn, C.H. (1995). Analysis of auxin and cytokinin activity of commercial Aqueous Seaweed Extract. *Gartenbauwissenschaft* 60(4):191-194.

Kaya, M., Atak, M., Çiftçi, C.Y., Ünver, S. (2005). Çinko ve hümik asit uygulamalarının ekmeklik buğday (*Triticum aestivum* L.)' da verim ve bazı verim öğeleri üzerine etkileri. *Fen Bilimleri Enstitüsü Dergisi* 9-3.

Kolsarıcı, Ö., Kaya, M.D., Day, S., İpek, A., Uranbey, S. (2005). Farklı humik asitdozlarının ayçiçeğinin (*Helianthus annuus* L.) çıkış ve fide gelişimi üzerine etkileri, *Akdeniz Üniversitesi Ziraat Fakültesi Dergisi,* 18 151.

Özenç, D.B., Şen, O. (2017). Farklı gelişim dönemlerinde uygulanan deniz yosunu gübresinin domates bitkisinin gelişim ve bazı kalite özelliklerine etkisi. *Akademik Ziraat Dergisi* (6): *Special issue:*235-242.

Özenç, D.B., Şenlikoğlu G. (2017). Kompost ve azotlu gübre uygulamasının ıspanak bitkisinin (*Spinacia oleracea* L.) gelişimi üzerine etkileri. *Akademik Ziraat Dergisi* (6) *Special issue:*:227-234.

Rauthan, B.S., Schnitzer, M. (1981) Effects of a soil fulvic acid on the growth and nutrient content of cucumber (*Cucumis sativus*) plants. *Plant Soil* 63:491–495.

Sasikala, M., Indumathi, E., Radhika, S., Sasireka, R. (2016). Effect of seaweed extract (Sargassum tenerrimum) on seed germination and growth of tomato plant (*Solanum lycopersicum*). *International Journal of ChemTech Research CODEN (USA):* IJCRGG, (9) No.09. 285-293.

Senesi, N., Loffredo, E., Padonava, G. (1990). Effects of humic acid. herbicide interactions on the growth of Pisum sativum in nutrient solution. *Plant and Soil* 127: 41–47.

Tahir, M., Khurshid, M., Khan, M., Z., Abbasi, M., K., Hazmi, M., H. (2011) Lignite-derived humic acid effect on growth of wheat plants in different soils. *Pedosphere* 2:124–131.

Tüzel, Y., Öztekin G. B., Duyar H., Eşiyok D., Kılıç G. Ö., Anaç D., Kayıkçıoğlu, H.H. (2011). Organik salata-marul yetiştiriciliğinde agryl örtü ve bazı gübrelerin verim, kalite, yaprak besin madde içeriği ve toprak verimliliği özelliklerine etkileri. *Tarım Bilimleri Dergisi* 17:190-203.

Verkleij, F.N. (1992). Seaweed extracts in agriculture and horticulture: *Biological Agriculture and Horticulture,* 8: 309-324.

Yılmaz, G.F., Harmankaya, M., Gezgin, S. (2012). Farklı demir bileşikleri ve TKİ-hümas uygulamalarının ıspanak bitkisinin demir alımı ve gelişimine etkileri. Türkiye 1. Ulusal Hümik Madde Kongresi 06-09 Haziran, Sakarya.

CHAPTER 5

BIOLOGICAL ACTIVITIES OF *GALANTHUS* SPECIES

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1. INTRODUCTION

The bulbous tuberous plants that spend most of the year underground with their organs such as onions, tubers and rhizomes and constitute an important part of floristic richness are called geophytes (ground plants) or cryptopythes (hidden plants) (Özuslu and İskender 2009). The word Geophyta is created by the union the words ‘geo’ meaning earth and ‘phyta’ designing plant and means ground plants or hidden plants. Geophytic plants are generally included in the families *Amaryllidaceae, Iridaceae, Liliaceae, Ranunculaceae, Primulaceae, Araceae, Geraniaceae and Orchidaceae* (Meerow, 2013). These families include economic importance, medicinal value, potential for use as an ornamental plant, and important species used in many large industries (Ay et al., 2018).

Galanthus species belonging to the *Amaryllidaceae* family are perennial, bulbous, herbaceous plants. *Galanthus* genus belonging to *Amaryllidaceae* family consists of 22 species, 8 varieties and 4 hybrids (World Cheklist of Selected Plant Families, 2020).

Galanthus species are commonly known as ‘snowdrops’. Snowdrop is an ornamental plant that is admired and preferred in Europe and many other countries because of its white, elegant and showy flowers blossom in winter and early spring. It is widely used in botanical gardens, landscaping applications in parks and stone gardens and as an ornamental plant in private gardens and is used in folk medicine to treat headache, and migraine. It is also considered as a medicinal plant due to some important alkaloids it contains.

Snowdrop contains important bioactive compouds such as phenolics, flavonoids, and terpenoids. These important bioactive compounds have strong antioxidant activity. Antioxidants block free radicals, minimize the damage they cause to the body, and prevent chain reactions that lead to premature aging as well as many diseases. Natural antioxidants commonly found in plants are phenolic compounds (tocopherols, flavonoids, and phenolic acids), nitrogen compounds (amino acids, amines, chlorophyll derivatives and alkaloids), ascorbic acid, carotenoids, polyphenols and selenium (Yeow et al., 2020). Many natural antioxidants display a broad range of biological effects, comprehending anti-inflammatory, antithrombotic, antibacterial, antiviral, and antiallergic properties (Aytar and Kömpe, 2021).

Amaryllidaceae alkaloids are a group of secondary metabolites that have been extensively studied due to their biogenesis, and pharmacological and physiological activities. Studies conducted on *Galanthus*, which was used mostly as an ornamental plant, have revealed that these plants are also rich in alkaloid content (Berkov et al., 2012; Kintsurashvili and Vachnadze, 2007; Ay et al., 2018; Batı Ay et al., 2020). The species included in the Amaryllidaceae family, in which the snowdrop is located, includes up to 150 alkaloids called Amaryllidaceae alkaloids. Amaryllidaceae type alkaloids are morphologically separated into 9 main groups: haemanthamine, lycorine, crinine, narciclasine, galanthamine, Tazettine, homolycorine, montanine, and norbelladine (Ferdausi, 2017). Some alkaloids of *Galanthus* species are used in treatment today. Galantamine and lycorine are the most important alkaloids found in the snowdrop plant, which have high biological activity and are especially found in onions (Janssen and Schäfer, 2017).

Galantamine is used in the treatment of neurological disorders, especially polio and Alzheimer's disease (AsD) (Unver, 2007). Galantamine acts on cholinergic neurotransmission in the brain through two different mechanisms. At low doses, it allosterically binds to nicotinic acetylcholine receptors (nAChRs) and activates their functions, while at high doses acetylcholinesterase (AChE) shows inhibitory activity (Maelicke et al., 2000). As a result of many studies, it has been determined that galantamine eases synaptic transmission (Schilstrom et al., 2007), shows a neuroprotective effect by preventing apoptosis and oxidative damage, increases learning ability under various experimental conditions, and is especially effective against cerebral ischemia (Lorrio et al., 2007). It has been reported that it inhibits the aggregation of β-amyloid plaques, which has a role in the pathogenesis of AsD, and also significantly reduces oxidative stress and cellular apoptosis induced by β-amyloid 1-40 (Matharu et al., 2009). In addition, clinical studies have shown that galantamine has positive effects on impaired dopaminergic neurotransmission in schizophrenia and may be beneficial in relieving some symptoms such as speech disorder in this disease (Schilstrom et al., 2007; Conley et al., 2009).

Another common alkaloid in *Galanthus* species, which has been extensively studied in terms of pharmacology, is the alkaloid called lycorine (Batı Ay et al., 2018, 2019, 2020). Lycorine is known to be a potential inhibitor of the ascorbic acid synthesis of higher plants, algae and yeasts, of cell growth and division and organogenesis, as well as inhibits the cell cycle during interphase (Bastida et al., 2006). It has been shown that lycorine alkaloid has antiviral (Li et al., 2005), antimalarial (Cedrón et al., 2010; Van Dyk et al., 2009), antifungal (Shen et al., 2014), hepatoprotective (Çitoğlu et al., 2012; Ilavenil et al. al., 2012) and antitumor (Li et al., 2012) activities. In addition to its analgesic (Çitoğlu et al., 2012) effect, lycorine has been reported to have a strong cardiotonic effect *in vitro* (Abdallah., 1993). In addition to these, there are studies showing the interaction of lycorine with DNA and/or RNA using different analysis methods (Hohmann et al., 2002). In some studies, it has been shown that it is effective against the paradise group called Trichomonas vaginalis, which is the causative agent of trichomoniasis (Giordani et al., 2012).

Apart from these two important alkaloids, there are also Amaryllidaceae alkaloids with different biological activities. For example, buphenamine, hypoeastrin, klivimin, trispheridin, 2-Oacetyllicorin, pretazettin, pancratistatin, narciclasin, 7-deoxynarcycline (lycoricidin), crinamine, narciprimine, arolicoricidin, bulbispermin, lycorenan-7-one, and hamentamine are anti-Amaryllide compounds that show activity with various mechanisms by various anti-Amaryllide compounds called amaryllids (Evidente et al., 2009; Sarikaya et al., 2012). Tazettin, hypoeastrin, hemantidine have been found to have cytotoxic activity against some tumor types, including P-388 murine lymphocytic leukemia (Antoun et al., 1993). In addition, hemantamine, dihydrolycorine, narcidin and sanguine have antiprotozoal effects (Kaya et al., 2011; Osorio et al., 2010). Hipeastrin, 11-hydroxyvittatin and hemantamine are alkaloids with antiviral effects. (He et al., 2012). Norbelladine has antioxidant and anti – inflammatory effects (Park., 2014). Finally, 6-hydroxyhamentamine and hamentamine are alkaloids with antimalarial activity (Cedrón et al., 2012).

In this research, it is aimed to discuss the secondary metabolites and their biological activities by reviewing the literature of *Galanthus* species.

2. BIOLOGICAL ACTIVITIES OF *GALANTHUS* SPECIES

Traditional medicine has been developing for centuries and has a wide range of applications. In particular, the biological activities of Galantamine and lycorine alkaloids such as antimicrobial, antioxidant and anticancer have recently attracted the attention of scientists, and the galantamine alkaloid isolated from snowdrop species has allowed it to be used as an active drug in the treatment of moderate Alzheimer's disease. In this study, studies on the biological activities of *Galanthus* species will be focused on, and anticholinesterase will be evaluated under separate headings as antimicrobial, antiviral, antioxidant and anticancer activity.

Anticholinesterase activity

The use of cholinesterase inhibitors as the first drug therapy for Alzheimer's disease has been proven by the US Food and Drug Administration (FDA). There are two cholinesterase enzymes in the body, acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) (Hartman, 2010). Acetylcholinesterase (AChE) is one of the main enzymes involved in the pathogenesis of AsD. In the normal adult brain, AChE is widely present, while BChE is present in limited amounts (Demans, 2002). The enzyme acetylcholinesterase is found in all excitable tissues. Butyrylcholinesterase enzyme is found in the central and peripheral nervous system, liver and plasma. BChE levels are high in the early stages of nervous system development, and this level decreases in the later stages. Galantamine is known to increase the activity of acetylcholine (ACh) by inhibiting the AchE enzyme and acting as a nicotinic activator by interacting with nicotinic ACh receptors (nAChRs) in the brain. The interaction between the Ach inhibitor and nAChR induces a conformational change of the receptor molecule, and subsequent activation of nAChRs is believed to have protective effects against β-amyloid cytotoxicity of neuron cells. Snowdrop plant is an important source of galantamine. Due to the restricted number of medicates effective for the treatment of AsD, significant efforts have been devoted to AChE inhibitor research from medicinal plants (Khaw et al., 2014, 2020; Tan et al., 2014; Jamila et al., 2015; Liew et al., 2015).

There are many studies conducted to determine the anticholinesterase activities of *G. elwesii, G. cilicicus G. nivalis*, *G. ikariae*, *G. rizehensis*, *G. xvalentinen and G. gracilis*. In a study, it was reported that the methanol extract of *G. nivalis* had 96% inhibition against AChE (Rhee et al., 2003). In similar studies, it was determined that the above-ground and onion methanol extracts of *Galanthus elwesii* inhibited AChE. (Bozkurt et al., 2013; Kaya et al., 2017). Subsequent GC-MS analysis revealed the presence of Galantamine, O-methylleukotamine, hordenine and sanguine alkaloids in *G. elwesii* extracts. (Bozkurt et al., 2017). It was reported that alkaloid extracts moderately inhibited AChE (IC50: 11.82–25.5 µg/ml) (Sarikaya et al., 2013; Bozkurt-Sarikaya et al., 2014). AChE and BuChE cholinesterase inhibitor values of *G. krasnovii* onion alkaloid were determined to be IC50: 8.26, 6.23 µg/ml, respectively. According to the GCMS analysis results, it was reported that the dominant compounds contributing to the inhibitory activity in onion extracts were anhydrolycorine and 11,12 didehydroanhydrolycorine (Bozkurt et al., 2020).

The results show that alkaloids from *Galanthus spp.* have an important role in cholinesterase inhibitory activity. The alkaloid content in onions appears to be higher in the alkaloid content of the aerial parts. Considering that current drugs are effective in mild to moderately advanced stages of AsD and have significant side effects, it is imperative to search for selective cholinesterase inhibitors with minimal side effects. In the light of all this information, it can be concluded that the bulbs of *Galanthus spp*. can be used as a source of anticholinesterase alkaloids.

Antimicrobial activity

The use of plants in the treatment of common infectious diseases begins in ancient times. Some of the medicines prepared from plants in traditional ways are still part of the customary treatment of various diseases. However, today, plants and herbal medicine raw materials constitute 25% of prescription drugs. The inadequacy of synthetic drugs against increasing diseases in recent years and the detection of their side effects have increased the necessity of using natural products. Especially recently, due to the increase in microorganisms with multiple antibiotic resistance, the treatment of infection caused by these microbes is becoming increasingly difficult. In bacteria that develop resistance to all known antibiotics, drug resistance is increasing and spreading. Therefore, it is very promising that medicinal plants can be used as new drug candidates as an alternative to drugs in the treatment of antibiotic resistant infections. In a study, the chemical content, antioxidant and antimicrobial properties of the essential oil of the aerial parts of *G. ikariae* Baker were investigated. The antimicrobial activity of the essential oil was studied against 12 bacteria and 5 yeast-molds by agar-well method and it was found to be particularly effective against Listeria monocytogenes bacteria and Saccharomyces cerevisiae yeast. (Üçüncü et al., 2019).

In a study with *G. transcaucasicus* from *Galanthus* species, methanol extracts of the plant were found to be effective against *B. subtilis, B. cereus, S. aureus, E. coli and P. aeruginosa* bacteria. It has been stated that the high antimicrobial activity of this plant is due to the phenolics, flavonoids and volatile compounds it contains, as well as the difference in microbial basis. (Karimi et al., 2018).

Antiviral activity

Viruses continue to be an important cause of morbidity and mortality worldwide, as in the past. They can cause very serious deadly diseases such as common infections such as flu, AIDS (acquired immune deficiency syndrome), Ebola, SARS (severe acute respiratory syndrome), some types of cancer and Covid-19 that we have been living with for the last few years. It is very difficult to develop safe and effective antiviral drugs against viruses. Classical antiviral drugs such as interferon and ribavirin are highly effective against most viruses *in vitro*. However, the same effect cannot be seen when applied to patients. As standard therapies for the management and control of viral infections are inadequate, there is a need to discover effective new antivirals. For these reasons, rational phytotherapy applied with natural, standardized herbal sources may be a suitable option for the treatment of viral diseases and the discovery of new natural origin antiviral active compounds.

Lectin derived from snowdrops is under investigation for its antiviral activity. In a study, *G. nivalis* agglutinin (GNA) was purified and identified from snowdrop bulbs (Van Damme et al., 1987). GNA is known to have virucidal properties against human immunodeficiency virus (HIV) EC50 0.12 ± 0.07 µg/ml to 4.7 ± 3 µg/ml (Balzarini et al., 2004). In a study to determine the effect of ethanol extracts of *G. elwesii* on anti-herpes simplex virus (HSV) and anti-sindbis virus (SINV) activity, *G. elwesii* activity in the virucidal (8 µg/ml) assay than the plaque. -forming assay (24 µg/ml) has been reported to have higher activity (Hudson et al., 2000).

In a study conducted to determine the antiviral activity of plant lectins from a collection of medicinal plants on feline infectious peritonitis virus (FIPV) and infected cells, it has been reported that plants derived from mannose-binding lections have the strongest anti-coronavirus activity and *Galanthus nivalis* is one of the plants that inhibit coronavirus. (Adams, 2020).

As a result, lectin GNA may be an option for naturally derived antiviral agents obtained from herbal sources in the treatment of viral diseases.

Antioxidant activity

Interest in natural antioxidants, especially found in fruits and vegetables, has been increasing among consumers and scientists in recent years. In addition to fruit and vegetable-based antioxidants, there are medicinal plants that can exhibit potent antioxidant activities. Antioxidant activity is a very important issue for human life and biological functions such as antimutagenic, anticarcinogenic and antiaging occur as a result of antioxidant activity (Haung et al., 1992; Cook and Samman, 1996). The active ingredients contained in snowdrop species, block the free radicals, minimize the damage they cause to the body and have an antioxidant effect that prevents chain reactions that lead to premature aging as well as many diseases.

Numerous studies have been conducted on this subject. (Ay et al., 2018) conducted a study to determine the total phenolic and total flavonoid content and antioxidant activity of *G. elwesii* in different plant organs (flower, leaf, root, onion) and different growth periods (flowering beginning and fruit ripening). When compared as the development period, fruit ripening was determined as > after flowering > beginning of flowering. When the antioxidant activity was compared according to different plant organs, remarkable differences were found between leaves, bulbs, roots and flowers; leaf > flower > onion > root. In a study to determine the antioxidant potential of the aerial parts and bulbs of *G. reginaeolgae*, aerial parts and onion extracts were reported to have moderate antioxidant activity (Conforti et al., 2010). In another study, DPPH and ABTS radical scavenging and copper ion reducing power methods were used to determine the antioxidant activities of hexane, dichloromethane and ethyl acetate extracts of *G. krasnovii*. It was determined that the dichloromethane extract showed the highest ABTS radical scavenging activity (IC50: 14.33 μg/ml) (Erenler et al., 2019).

Snowdrop species, which are rich in secondary metabolites such as phenolic acids, flavonoids and alkaloids, appear to be a strong antioxidant source. Secondary metabolites from *Galanthus spp.* species can reduce the risk or slow the progression of many diseases.

Anticancer activity

Cancer is a chronic disease that kills millions of people each year (Tan et al., 2016; Tay et al., 2019). Cancerous cells are cells that have ability to multiply uncontrollably. Chemotherapy, radiotherapy and chemically derived drugs are used during the treatment of cancer. Patients treated with chemotherapy may be subject to a great deal of strain, further detrimental to one's health. For this reason, the importance of alternative treatments and therapies against cancer, especially of natural origin, is increasing (URL-4, 2018). New cellular targets and anticancer agents of natural origin are needed.

It has been determined that onion extracts from methanol onion and aerial parts extracts obtained from a worldwide successful *Galanthus* species in terms of searching for new anticancer agents from natural sources show higher cytotoxic activity. They reported that *G. platyphyllus* bulbs are more active against Human promyelocytic leukemia cells (Hela) cells than other cell lines, as well as more active against the majority of the species, Human colorectal carcinoma cells (HCT-116). The most cytotoxic (IC50 < 10 µg/ml) onions in HCT-116 cells were found to be *G. krasnowii, G. woronowii, G. alpinus and G. shaoricus*. While lycorine was cytotoxic to HCT-116, HL-60 and Hela cells with an IC50 of 8.2 and 9.3 µM, galantamine and freshwater showed weak cytotoxic activity against HCT-116, HL-60 and Hela cells with IC50 > 100 µM (Jokhadze et al., 2007).

Due to the resistance to these drugs in treatments with chemotherapy drugs, it is thought that natural-source alternative treatments and therapies will make a great contribution to science in the fight against cancer.

3. CONCLUSION

Although there are new drugs that come with developing technology and newly produced treatment techniques since the dangerous side effects of these drugs cannot be ignored, interest in scientific studies on natural products has increased and research on their bioactive properties has deepened. Plants contain a significant number of phytochemical components, most of which are known to be biologically active and responsible for various pharmacological activities. It has shown by studies that these compounds have the potential to provide people to live a healthier life in the aspects of its usage owing to antioxidants properties instead of synthetic antioxidants, in preventing the development of pathogenic microorganisms with their antimicrobial properties, usage in inflammation with their anti-inflammatory properties, in reducing the risk and treatment of cancer with their anticancer properties, and in preventing the harmful effects of free radicals that may occur in the later stages of life. Considering the significant number of phytochemicals that exists in plants, *Galanthus spp*. which has quite different biological activities, can be considered as a natural product score. This review highlights the importance of various secondary metabolites of *Galanthus spp*. on anticholinesterase inhibitory activity and some other diseases. *Galanthus spp*. Although galantamine and lycorine show more activity than other alkaloids in species, their mechanism of action and the number of studies on these bioactive substances are very few. Apart from the activities mentioned in the study, for the widespread use of snowdrop in various treatments, its ethnopharmacological uses need to be proven with strong scientific studies.

In addition, further isolation, identification, mechanism of action and synthetic studies of existing and novel active compounds are required to better understand the basis of activity at the cellular and molecular level of *Galanthus spp*. For all these reasons, this review may serve as a guide for future researchers to conduct further studies by providing a different perspective on these plants.

REFERENCES

Abdallah, O. M. (1993). Narcisine, an alkaloid from Narcissus tazetta. *Phytochemistry* 34(5): 1447-1448.

Adams, C. (2020). Can red algae and mannose-binding lectins fight coronavirus (COVID-19). *J. Plant Med*.

Antoun, M. D., Mendoza, N. T., Ríos, Y. R., Proctor, G. R., Wickramaratne, D. M., Pezzuto, J. M., Kinghorn, A. D. (1993). Cytotoxicity of Hymenocallis expansa alkaloids. *Journal of Natural Products*, 56(8): 1423-1425.

Avcu, C. (2011). Morphological and ecological studies on Mount Katra (Çanakale/Bayramiç) and its surrounding geophytic plants. Balıkesir University Institute of Science and Technology.

Ay, B.E., Gül, M., Açikgöz, M.A., Yarilgaç, T., Kara, Ş.M. (2018). Assessment of Antioxidant Activity of Giant Snowdrop (*Galanthus elwesii* Hook) Extracts with Their Total Phenol and Flavonoid Contents. *Indian Journal of Pharmaceutical Education and Research* 52(4): 128-132.

Aytar, E.C., Kömpe, Y.Ö. (2021). Traditional Uses, Phytochemical Contents and Biological Activities of Orchids. *Black Sea Journal of Engineering and Science* 9-10.

Balzarini, J., Hatse, S., Vermeire, K., Princen, K., Aquaro, S., Perno, C. F., ... Schols, D. (2004). Mannose-specific plant lectins from the Amaryllidaceae family qualify as efficient microbicides for prevention of human immunodeficiency virus infection. *Antimicrobial agents and chemotherapy* 48(10): 3858-3870.

Bastida, J., Lavilla, R., Viladomat, F. (2006). Chemical and biological aspects of Narcissus alkaloids. *The Alkaloids: Chemistry and Biology* 63: 87-179.

Başköse, I., Paksoy, M.Y., Selvi, S. (2012). Geophytic plants around the Akkaya dam lake (Nigde-Türkiye). In *XI International Symposium on Flower Bulbs and Herbaceous Perennials* 1002:43-47.

Batı Ay, E., Gül, M., Kocaman, B., Açıkgöz, M.A. (2019). Photophysical characterization of *Galanthus elwesii* hook. *International Journal of Science Letters* 1(1): 20-29.

Batı Ay, E., Açıkgöz, M.A., Kocaman, B., Kara, Ş.M. (2020). Comparative study of maceration and ultrasonic-assisted extraction of galantamine and lycorine content and antioxidant activity of *Galanthus elwesii* Hook. *Academic Journal of Agriculture* 9(2): 297-306.

Berkov, S., Codina Mahrer, C., Bastida Armengol, J. (2012). The genus *Galanthus*: A source of bioactive compounds. in: Rao, Venketeshwe. Phytochemicals: A Global Perspective of Their Role in Nutrition and Health. IntechOpen.

Bozkurt, B., Kaya, G., Önür, M., Bastida, J., Somer, N. (2013). Phytochemical investigation of *Galanthus woronowii*. *Biochem. Systemat. Ecol*. 51: 276–279.

Bozkurt, B., Coban, G., Kaya, G.I., Onur, M.A., Unver-Somer, N. (2017). Alkaloid profiling, anticholinesterase activity and molecular modeling study of *Galanthus elwesii*. *South African Journal of Botany* 113: 119-127.

Bozkurt, B., Kaya, G.I., Onur, M.A., Unver-Somer, N. (2021). Chemo-profiling of some Turkish *Galanthus* L. (*Amaryllidaceae*) species and their anticholinesterase activity. *South African Journal of Botany* 136: 65-69.

Bozkurt-Sarikaya, B., Kaya, G.I., Onur, M.A., Bastida, J., Berkov, S., Unver-Somer, N. (2014). GC/MS analysis of *Amaryllidaceae* alkaloids in *Galanthus gracilis*. *Chemistry of Natural Compounds* 50(3): 573-575.

Cedrón, J.C., Gutiérrez, D., Flores, N., Ravelo, Á.G., Estévez-Braun, A. (2010). Synthesis and antiplasmodial activity of lycorine derivatives. *Bioorganic & medicinal chemistry* 18(13): 4694-4701.

Conforti, F., Loizzo, M.R., Marrelli, M., Menichini, F., Statti, G.A., Uzunov, D., Menichini, F. (2010). Quantitative determination of *Amaryllidaceae* alkaloids from *Galanthus reginae-olgae* subsp. *vernalis* and *in vitro* activities relevant for neurodegenerative diseases. *Pharmaceutical Biology* 48(1), 2-9.

Conley, R.R., Boggs, D.L., Kelly, D.L., McMahon, R.P., Dickinson, D., Feldman, S., ... Buchanan, R.W. (2009). The effects of galantamine on psychopathology in chronic stable schizophrenia. *Clinical Neuropharmacology* 32(2): 69.

Cook, N.C., Samman, S. (1996). Flavonoids—chemistry, metabolism, cardioprotective effects, and dietary sources. *The Journal of Nutritional Biochemistry* 7(2): 66-76.

Çitoğlu, G.S., Acıkara, Ö.B., Yılmaz, B.S., Özbek, H. (2012). Evaluation of analgesic, anti-inflammatory and hepatoprotective effects of lycorine from *Sternbergia fisheriana* (Herbert) Rupr. *Fitoterapia* 83(1): 81-87.

Erenler, R., Nusret, G., Elmastaş, M., Eminağaoğlu, Ö. (2019). Evaluation of antioxidant capacity with total phenolic content of *Galanthus krasnovii* (*Amaryllidaceae*). *Turkish Journal of Biodiversity* 2(1):13-17.

Evidente, A., Kireev, A.S., Jenkins, A.R., Romero, A.E., Steelant, W.F., Van Slambrouck, S., Kornienko, A. (2009). Biological evaluation of structurally diverse *Amaryllidaceae* alkaloids and their synthetic derivatives: discovery of novel leads for anticancer drug design. *Planta medica* 75(05): 501-507.

Ferdausi, A. (2017). A metabolomics and transcriptomics comparison of Narcissus pseudonarcissus cv. Carlton field and in vitro tissues in relation to alkaloid production. University of Liverpool.

Giordani, R.B., Junior, C.O., de Andrade, J.P., Bastida, J., Zuanazzi, J.A., Tasca, T., de Almeida, M.V. (2012). Lycorine derivatives against Trichomonas vaginalis. *Chemical biology & drug design* 80(1): 129-133.

Güner, H.B. (2006). *Inventory of Turkish geophytes growing in botanical gardens in Istanbul*. (Master's Thesis), Istanbul University Institute of Science and Technology, İstanbul.

Hartman, R.E. (2010). Actions of Bioactive Phytochemicals in Cell Function and Alzheimer's Disease Pathology. Taylor & Francis Group, LLC chapter 16.

He, J., Qi, W.B., Wang, L., Tian, J., Jiao, P.R., Liu, G.Q., ... Liao, M. (2013). Amaryllidaceae alkaloids inhibit nuclear-to-cytoplasmic export of ribonucleoprotein (RNP) complex of highly pathogenic avian influenza virus H5N1. *Influenza and other respiratory viruses* 7(6): 922-931.

Hohmann, J., Forgo, P., Molnár, J., Wolfard, K., Molnár, A., Thalhammer, T., ... Sharples, D. (2002). Antiproliferative Amaryllidaceae alkaloids isolated from the bulbs of Sprekelia formosissima and Hymenocallis× festalis. *Planta medica* 68(05): 454-457.

Hudson, J.B., Lee, M.K., Sener, B., Erdemoglu, N. (2000). Antiviral activities in extracts of Turkish medicinal plants. *Pharmaceutical biology* 38(3): 171-175.

Ilavenil, S., Kaleeswaran, B., Ravikumar, S. (2012). Protective effects of lycorine against carbon tetrachloride induced hepatotoxicity in Swiss albino mice. *Fundamental & clinical pharmacology* 26(3): 393-401.

Janssen, B., Schäfer, B. (2017). Galantamine. *ChemTexts* 3(2): 1-21.

Jamila, N., Khairuddean, M., Yeong, K.K., Osman, H., Murugaiyah, V. (2015). Cholinesterase inhibitory triterpenoids from the bark of Garcinia hombroniana. *Journal of Enzyme Inhibition and Medicinal Chemistry* 30(1): 133-139.

Jokhadze, M., Eristavi, L., Kutchukhidze, J., Chariot, A., Angenot, L., Tits, M., ... Frédérich, M. (2007). In vitro cytotoxicity of some medicinal plants from Georgian *Amaryllidaceae*. Phytotherapy Research: *An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives* 21(7): 622-624.

Karimi, E., Mehrabanjoubani, P., Homayouni-Tabrizi, M., Abdolzadeh, A., Soltani, M. (2018). Phytochemical evaluation, antioxidant properties and antibacterial activity of Iranian medicinal herb *Galanthus transcaucasicus* Fomin. *Journal of Food Measurement and Characterization* 12(1): 433-440.

Kaya, G.I., Uzun, K., Bozkurt, B., Onur, M.A., Somer, N.U., Glatzel, D.K., Fürst, R. (2017). Chemical characterization and biological activity of an endemic *Amaryllidaceae* species: *Galanthus cilicicus*. *South African Journal of Botany* 108: 256-260.

Khaw, K.Y., Choi, S.B., Tan, S.C., Wahab, H.A., Chan, K.L., Murugaiyah, V. (2014). Prenylated xanthones from mangosteen as promising cholinesterase inhibitors and their molecular docking studies. *Phytomedicine* 21(11): 1303-1309.

Khaw, K.Y., Chong, C.W., Murugaiyah, V. (2020). LC-QTOF-MS analysis of xanthone content in different parts of Garcinia mangostana and its influence on cholinesterase inhibition. *Journal of enzyme inhibition and medicinal chemistry* 35(1): 1433-1441.

Kintsurashvili, L., Vachnadze, V. (2007). Plants of the *Amaryllidaceae* family grown and introduced in Georgia: a source of galanthamine. *Pharmaceutical Chemistry Journal* 41(9): 492-494.

Liew, S.Y., Khaw, K.Y., Murugaiyah, V., Looi, C.Y., Wong, Y.L., Mustafa, M.R., ... Awang, K. (2015). Natural indole butyrylcholinesterase inhibitors from *Nauclea officinalis*. *Phytomedicine* 22(1): 45-48.

Li, S.Y., Chen, C., Zhang, H.Q., Guo, H.Y., Wang, H., Wang, L., ... Tan, X. (2005). Identification of natural compounds with antiviral activities against SARS-associated coronavirus. *Antiviral Research* 67(1): 18-23.

Lorrio, S., Sobrado, M., Arias, E., Roda, J.M., García, A.G., Lopez, M.G. (2007). Galantamine postischemia provides neuroprotection and memory recovery against transient global cerebral ischemia in gerbils. *Journal of Pharmacology and Experimental Therapeutics* 322(2): 591-599.

Maelicke, A. Albuquerque, E.X. (2000). Allosteric modulation of nicotinic acetylcholine receptors as a treatment strategy for Alzheimer's disease. *European Journal of Pharmacology* 393(1-3): 165-170.

Matharu, B., Gibson, G., Parsons, R., Huckerby, T.N., Moore, S.A., Cooper, L.J., ... Austen, B. (2009). Galantamine inhibits β-amyloid aggregation and cytotoxicity. *Journal of the Neurological Sciences* 280(1-2): 49-58.

Meerow, A.W. (2013). Taxonomy and phylogeny. In: Kamenestky R, Okubo H, Eds. Ornamental geophytes: from basic science to sustainable production. Boca Raton: CRC Press.

Osorio, E. J., Berkov, S., Brun, R., Codina, C., Viladomat, F., Cabezas, F., Bastida, J. (2010). *In vitro* antiprotozoal activity of alkaloids from *Phaedranassa dubia* (*Amaryllidaceae*). *Phytochemistry Letters* 3(3): 161-163.

Özuslu, E. İskender, E. (2009). Bulbous Plants of Mount Sof (Gaziantep), Biological Diversity and Conservation, BioDiCon 2/2: 78-84.

Park, J.B. (2014). Synthesis and characterization of norbelladine, a precursor of *Amaryllidaceae* alkaloid, as an anti-inflammatory/anti-COX compound. *Bioorganic & Medicinal Chemistry Letters* 24(23): 5381-5384.

Rhee, I.K., Appels, N., Luijendijk, T., Irth, H., Verpoorte, R. (2003). Determining acetylcholinesterase inhibitory activity in plant extracts using a fluorimetric flow assay. Phytochemical Analysis: *An International Journal of Plant Chemical and Biochemical Techniques* 14(3): 145-149.

Sarikaya, B.B., Kaya, G.I., Onur, M.A., Viladomat, F., Codina, C., Bastida, J., Somer, N.U. (2012). Alkaloids from *Galanthus rizehensis*. *Phytochemistry Letters* 5(2): 367-370.

Sarikaya, B.B., Berkov, S., Bastida, J., Kaya, G.I., Onur, M.A., Somer, N.U. (2013). GC-MS Investigation of *Amaryllidaceae* alkaloids in *Galanthus* x *valentinei notho* subsp. *subplicatus*. *Natural Product Communications* 8(3): 1934578X1300800312.

Shen, J.W., Ruan, Y., Ren, W., Ma, B.J., Wang, X.L., Zheng, C.F. (2014). Lycorine: a potential broad-spectrum agent against crop pathogenic fungi. *Journal of Microbiology and Biotechnology* 24(3): 354-358.

Schilström, B., Ivanov, V.B., Wiker, C., Svensson, T.H. (2007). Galantamine enhances dopaminergic neurotransmission *in vivo* via allosteric potentiation of nicotinic acetylcholine receptors. *Neuropsychopharmacology* 32(1): 43-53.

Tan, W.N., Khairuddean, M., Wong, K.C., Khaw, K.Y., Vikneswaran, M. (2014). New cholinesterase inhibitors from Garcinia atroviridis. *Fitoterapia* 97: 261-267.

Tan, H.L., Chan, K.G., Pusparajah, P., Saokaew, S., Duangjai, A., Lee, L.H., Goh, B.H. (2016). Anti-cancer properties of the naturally occurring aphrodisiacs: icariin and its derivatives. *Frontiers in Pharmacology* 7: 191.

Tay, K.C., Tan, L.T.H., Chan, C.K., Hong, S.L., Chan, K.G., Yap, W.H., ... Goh, B.H. (2019). Formononetin: a review of its anticancer potentials and mechanisms. *Frontiers in Pharmacology* 10: 820.

Unver, N. (2007). New skeletons and new concepts in *Amaryllidaceae* alkaloids. *Phytochemistry Reviews* 6(1): 125-135.

Üçüncü, O., Baltaci, C., Karataş, Ş.M., Muslu, A., Büyükçekiç, D., Ejderha, H., Özdemir, E.E. (2019). Chemical composition and biological activities of the essential oil of the above ground parts of *Galanthus ikariae* Baker. *Gümüşhane University Journal of Science* 9(4): 674-680.

Van Dyk, S., Griffiths, S., van Zyl, R.L., Malan, S.F. (2009). The importance of including toxicity assays when screening plant extracts for antimalarial activity. *African Journal of Biotechnology* 8(20).

Yeow, L.C., Chew, B.L. Sreeramanan, S. (2020). Elevation of secondary metabolites production through light-emitting diodes (LEDs) illumination in protocorm-like bodies (PLBs) of Dendrobium hybrid orchid rich in phytochemicals with therapeutic effects. *Biotechnology Reports* 27: e00497.

World Checklist of Selected Plant Families (2020). World checklist of *Galanthus*. Kew.: Facilitated by the Royal Botanic Gardens.

CHAPTER 6

PLANT GROWTH TIME AND YIELD RELATIONS IN VEGETABLES

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1. INTRODUCTION

Green plants containing chlorophyll produce their nutrients by combining water and carbon dioxide with light energy and releasing oxygen (Gaunt, 1995; Taiz and Zeiger, 2008). Leaves and other photosynthetic organs act as solar energy collectors and gas exchangers (Hay and Walker, 1989). Consequently, plants 'having green leaves for a longer period' helps them to photosynthesize for a longer time, and increase total yield (Ellis et al., 1990; Uzun, 2000). In the light of this approach, we can list the main determinants of yield as follows;

1. The amount of light cut off by the plant leaves while the dry matter accumulates in the harvested parts of the plant. This parameter should be evaluated together with the plant growth period, the anatomical structure of the leaf, and the canopy architecture. Studies have shown that increasing the leaf area index increases dry matter production and yield (Kaur and Singh, 2013).
2. The efficiency of converting the absorbed light into carbohydrates by photosynthesis after light cutting (Taiz and Zeiger, 2008). This parameter can be associated with the suitability of environmental conditions.
3. The rate of photosynthesis products transported to the harvested parts of plants. This parameter can be associated with environmental conditions and cultural practices (Uzun et al., 1998).
4. Weight loss by respiration and decay after photosynthesis and biosynthesis processes are completed. The difference between day and night temperatures should be associated with lighting, irrigation, plant nutrition and cultural practices.

5. Conversion coefficient between photosynthetic sugars and biochemical structures of harvested materials. This parameter can also change with environmental conditions, irrigation and fertilization. In this case, the longer the plant growth period (slow and stable growth), the greater the amount of light energy the plant leaves will cut, and accordingly, the yield elements that the plant will produce will increase (Hay and Walker, 1989).

2. FACTORS AFFECTING PLANT GROWTH TIME

We can list the factors affecting the plant growth period as follows;

- Plant canopy structure (architecture)
- Environmental factors (temperature, light)
- Variety characteristics (earliness, semi-earliness or late)
- Cultural practices (irrigation, pruning, fertilizing, managing the planting time)
- Quality of starting material (vegetable seeds or seedlings)

Plant canopy structure

Dry matter production of the plant and its distribution to the plant organs or the harvested parts of the plant can be affected by the plant canopy architecture-light relationships (Figure 1). Plant canopy architecture also indirectly influences photosynthesis, transpiration, pathogen infection, insect growth and reproduction, and photomorphogenesis (Acock et al., 1978; Hay and Walker, 1989). Environmental factors are as influential as the canopy architecture on the plant growth time.

Figure 1. Plant canopy architecture (Photo: Andaç Saka)

Environmental factors

The effects of environmental factors on the growth and development of various plant species in greenhouse systems are different. Plant growth and development could be controlled by controlling environmental factors in these systems. As it is known, environmental factors such as light and temperature play an important role in the course of basic physiological events in plants (Hay and Walker, 1989; Uzun and Demir, 1996; Uzun, 1996, 1997, 2001).

It is very important to understand the changes in yield in studies conducted to determine the effects of environmental conditions on plant growth. Considering the factors affecting photosynthesis, the relationships between light and temperature were studied (Kandemir and Uzun, 2019).

- **Temperature**

High temperatures have been observed to shorten plant growth period, decrease total yield, and provide earliness. It has also been stated that high temperatures cause a decrease in harvest index (the ratio of dry matter divided into yield components to total dry matter) and yield (Ellis et al., 1990). The decrease of harvest index is closely related to the plant canopy structure, and plant dry matter distribution is also affected. Studies have shown that high temperatures reduce the total yield of many plant species. The reason is generally accepted as high temperature shorten the plant growth period and increase the plant growth rate (Uzun, 2000).

In this case, some precautions could be taken to eliminate the negativities. For example, since fruit production in truss tomato varieties continues for a long time, vegetative structures should be balanced after the plant's transition to the generative stage. In other words, the assimilation rate produced by photosynthesis must be kept in balance during vegetative and generative growth. Because the maximum yield that can be obtained as a result of a long growth period depends on the balance achieved during the vegetative and generative development. Temperature significantly affects earliness in vegetables. Because the dry matter transported to the fruit at low temperatures is lower than that carried at higher temperatures. However, the increase in temperature decreased the average fruit sizes and weights and, thus, the total yield. Optimum growing temperature ranges were determined using plant growth models (Kürklü, 1998). Leaf and cluster formation rates decrease proportionally with decreasing temperature. Although these rates differ for the varieties, the response to temperature is the same. Went (1945) found that the optimum temperature required for growth rate decreased with plant age.

However, Ellis et al. (1990) showed that this decrease in growth rate was due to the decrease in relative growth rate and the optimum temperature remained constant. Young plants growing at optimal temperatures have thicker leaves and less instantaneous light-cutting surfaces, but since they will have long-term light-cutting abilities, the total amount of cut-off light will be high. Growth time may differ between non-contiguous plants and even among varieties of the same species (Ploeg and Heuvelink, 2005). High temperatures increase the leaf growth and expansion rate, as well as shorten the lifespan of the leaf and decrease their photosynthetic capacity much earlier than leaves exposed to lower temperatures (Figure 2). In this case, the total life span of the plant will be shortened and plants at lower temperatures will stay green longer and be able to photosynthesize (Uzun, 2000). Especially in vegetables whose leaves are consumed, high temperatures cause a bitter taste due to the loose leaf structure and yield losses (Dacoteau, 2000). It could be said that the plants grown at higher temperatures ripen early, but the overall yield will be less as the crop time will be short (Hay and Walker, 1989).

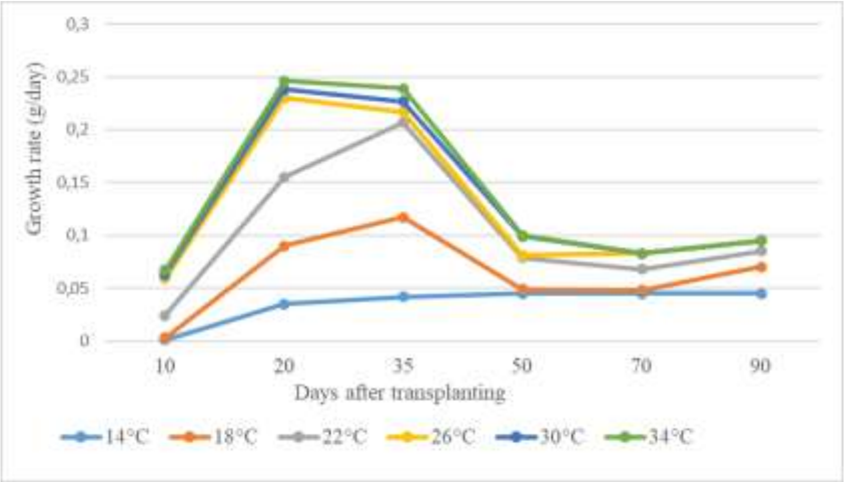

Figure 2. The relationship between the number of days after planting and the proportional growth rate

- **Light**

Plants need a certain amount of light for growth and development. Light is electromagnetic radiation or a quantum phenomenon subject to photons. It has been stated that light intensity accelerates growth and development in proportion to increase within a certain limit (Heuvelink, 1989; Uzun, 1996; Aybak, 2002). The duration of high-intensity light, which is interrupted by the plant when dry matter accumulation begins on the harvested parts, is also important, as well as absorbing light due to the interruption of the light by the leaves (Charles et al., 1986; Uzun, 2000).

It has been revealed in many studies that it increases the efficiency up to a certain intensity and then decreases it depending on the efficiency of the light being absorbed by the plant leaves and used in photosynthesis (Doaris et al., 1991; Uzun, 2000; Kurtar and Odabaş, 2010). It is known that high energy increases the leaf temperature, decreases the photosynthesis rate, and causes weight loss with respiration.

In a study investigating the effects of light on flower buds and fruit number in tomatoes, it was revealed that high light intensity (150 µmol $m^{-2} s^{-1}$) increased fruit number by 10% compared to low light intensity (100 µmol $m^{-2} s^{-1}$). Light has efficiency-enhancing effects from this point of view (Doaris et al., 1991). However, when examining the effect of light and temperature on yield, these two parameters should not be evaluated separately. Because the yield elements in plants show significant changes in most cases due to the interactive effects of light and temperature. High quality plant growth is obtained at different light intensities and between certain temperature degrees. It could be said that there is a positive relationship between temperature and light (Sarıbaş et al., 2017). It has been revealed that by controlling light and temperature in greenhouses, inflorescences and flowers that turn into fruits in vegetables can be controlled (Hay and Walker, 1989; Uzun, 2006; Uzun, 2007). Considering that other factors affecting plant growth are constant, increasing photosynthesis and decreasing respiration increase net assimilation and dry matter production (Uzun et al., 1998).

Variety traits

Today, selecting suitable varieties is very important in successful vegetable cultivation. Earliness or lateness should be considered in terms of plant growth times when choosing varieties. Plant growth times are shorter in early cultivars and longer in late cultivars. Plant growth times vary according to cultivar characteristics and yield also varies depending on the ripening time of cultivars.

Cultural practices

- **Pruning**

Pruning vegetables prevents unbalanced growth, provides better air circulation and lighting, and maintains yield. It also facilitates other cultural practices and harvesting. Leaves contaminated with diseases and pests with low photosynthetic capacity should be removed from the plants by pruning. Therefore, the leaves will aerate better and use light, making it possible to increase the total yield by enabling the plants to photosynthesize for a longer time. High dry matter content, fruit firmness, and yield were recorded in pruned plants compared to plants that were not pruned, in which pruning has significant effects on yield and quality characteristics of vegetables (Özdemir and Özer, 2015).

- **Soil structure and preparation**

The depth of light and heavy soils affects the growing period depending on their moisture, plant nutrients and soil conditioning elements. The growing period of vegetable species may vary according to soil requirements. In this case, the soil temperature may change according to its structure and affect plant growth, development, and yield.

- **Raised bed growing system**

First, the raised bed growing system has less labor and more weed control. In this system, plants do not enter each other's growing area; therefore, it eliminates competition with weeds and ensures stable growth (Figure 3).

Since weeds are deep from the surface in the raised bed growing systems, they cannot easily emerge and, therefore, cannot reach the raised bed. In this system, it is possible to extend the growing season, and it provides early warming of the soil and allows vegetation to continue until autumn. Since puddles that cause fungal diseases will seep into the soil in the tubes, diseases are prevented, and the growing season is extended (Özer, 2012).

Figure 3. Raised bed growing systems (Özer, 2012)

- **Mulching**

Culturing by covering the soil surface with natural or artificial materials in vegetable cultivation is called “mulching.” Using different mulching materials (organic, inorganic) provides some advantages in vegetable cultivation in terms of extending plant growth period, earliness, quality, and yield. Mulching application has emerged as an important factor in plant breeding in recent years. In the studies, plant growth periods have been extended significantly with mulching and yield has been increased thanks to the preservation of soil moisture, weed, disease and pest control, and reduction of water and nutrient losses (Kocer and Eltez, 2004; Radics et al., 2004; Khan et al., 2007; Zanic et al., 2009; Özer, 2012).

Some advantages of mulching are;

1. Conservation of soil water: Mulches reduce water loss from the soil surface and reduce irrigation needs.
2. Weed control: By preventing weed growth, mulches also prevent the struggle of weeds with the plants in terms of light, water, and nutrients (Figure 4).
3. Controlling temperature changes: As a general rule, organic mulches keep the soil warm, while light or colored plastic mulches increase the soil temperature. In this regard, organic mulches are recommended in temperate regions during summer. By using mulch, temperature fluctuations that affect vegetables negatively are also prevented.
4. Conservation and improvement of soil structure: Mulches eliminate the adverse effects of rain or irrigation systems that hit the soil. When organic mulches are applied, the lower parts of the mulch are broken

down and increasing the organic matter of the soil and indirectly improving the soil structure.

5. Reducing water and nutrient losses: When mulches are used, nutrient losses from the soil will be reduced in rains or excessive irrigation. On the other hand, using organic mulches could minimize the loss of water from the soil surface by rain or excessive irrigation and its inability to be taken by plants.
6. Reducing the insect population: It has been shown that plastic mulches (shiny, gilded, i.e. the upper part is shiny, the lower part is black, with aluminum strips) significantly reduced aphid contamination.
7. Preventing muddy water splashes on plant leaves during rain: It is important in terms of keeping plants cleaner and reducing the spread of diseases. On the other hand, it causes a decrease in the permeability of the leaf pores (i.e., a decrease in the rate of photosynthesis) and the spread of diseases.

Figure 4. Raised bed growing systems with mulch application (Anonymous, 2011)

- **Irrigation**

Irrigation is an important factor affecting vegetable yield (Al-Omran and Louki, 2011; Seidel et al., 2017; Parkash et al., 2021). If the irrigation is not enough during growth, stomata close, and CO_2 does not enter the leaf (Taiz and Zeiger, 2008). Photosynthesis declines rapidly, causing growth to slow down, resulting in a decrease in yield. Insufficient irrigation will reduce the yield. As a result of excessive irrigation, the plant will experience stress, so the photosynthesis capacity will decrease faster than expected as the plant grows early and age. In this case, the total yield will decrease and prevent the spread of the yield period over a long period.

- **Plant density**

Plant density causes the growth period, and high planting density has the least growth rate (Bleasdale, 1966). Since the dry matter content of individual plants will be low in dense plantings, the total yield will be low due to early maturation. The effects of plant population and planting spacing on yield are also related to other factors limiting plant growth (Fordham and Biggs, 1985; Saka, 2012). Dense planting causes lower yields compared to the plants planted in wide space, and there are decreases in the fruit number, average fruit weight, yield per plant, and dry matter content (Ganesan and Subbiah Vijay, 2004). Studies in vegetable species have revealed the importance of plant density adjustment (Khan et al., 2021; Sandhu et al., 2021; Mishra et al., 2022).

- **Seed sowing or seedling planting time**

As the growing period may be affected by early or suitable planting within the vegetation period for any vegetable species, an increase in yield is observed. The reason for that could be explained by the fact that when dry matter accumulation begins in the harvested organs of the plant, the plant has a certain leaf area earlier and will stay longer in sufficient light conditions. In a study conducted by Brewster (1994), when the effect of sowing date on head yield and light cutting of onions was examined, it was observed that the yield decreased if the seed sowing time was delayed (Table 1).

Table 1. The effect of seed sowing date on onion bulb yield and light cutting (Brewster, 1994)

Parameter	Seed planting dates	
	March 15	April 21
Head yield (kg/m^2 , dry matter)	1.04	0.73
Leaf area index during bulb formation	3.7	1.5
Percentage of light cut by leaves in bulb formation (%)	69	45

Quality of starting material (seed or seedling)

Today, using quality seedlings is important for successful and purposeful vegetable cultivation. Production of high quality seedlings in vegetables is one of the basic principles of successful production (Demir, 2007; Kandemir et al. 2013; Balkaya et al., 2015; Tüzel et al., 2015). The seedling must also be of good quality for a quality and long growing period, and seedlings should grow slowly and stable (Figure 5).

Figure 5. Example of slow and stable growth of vegetable seedlings (Uzun, 2008)

Factors such as light, temperature, humidity, irrigation, the physical condition of the seedling and the vitality level of the plant growing medium should also be taken into account. The advantages of seedling cultivation are free production from diseased and weak seedlings, homogeneity in plant growth and development, earliness, time and space saving, prevention of seed loss, control of seedling growth and productivity (Demir and Çakırer, 2015; Aktaş and Öztekin, 2019).

Growth is excessive in some cases in seedling cultivation. In such cases, some chemicals that are allowed to be used and that provide height control can be applied to the plants in appropriate doses so that the growth can be controlled; thus, quality plant growth is ensured. (Uğur and Kavak, 2007; Geboloğlu et al., 2015; Uçan and Uğur, 2021). Thus, since the plants will be more resistant to environmental conditions during the growing stages, they can pass this period without stress and increase their yield.

For maximum yield, the seedlings must not form fruits, flowers, or clusters before planting. An ideal seedling should be young, with homogeneous growth and high dry matter content. The planting process is followed by a rapid growth period under environmental conditions. Thus, healthy and sufficient growth is ensured before fruit formation (Anonymous, 2022).

3. PLANT GROWTH MODELS

Studies on the plant growth model aim to reveal different growth conditions by examining the effects of environmental conditions on plant growth. In these modeling studies, especially in developed countries, different plant growth models were revealed and the relationships between plant growth time and yield were investigated with these models (Uzun et al., 2001; Balkaya, 2004; Özkaraman, 2004; Kurtar and Odabaş, 2010; Sarıbaş, 2013; Demirsoy et al., 2016; Sarıbaş et al., 2017; Kandemir and Uzun, 2019; Yörük et al., 2021). In recent years, researchers have developed simulation models for different plant species. Models generally describe the plant growth, development, and yield characteristics (Tsuji et al. 1994).

In studies on modeling plant growth, events during plant development are taken into account and plant growth is simulated accordingly (Şaylan et al., 1998). Plant growth models are very important for production planning, and planned production is carried out by determining the intervals for optimum plant growth and optimum yield (Kürklü, 1998). In summary, plant growth models are used to explain the mathematical relationship between climate and soil conditions and predict plant physiology according to these conditions (Uzun, 1996; Pamuk and Özgürel, 2005). One of the important usage areas of the models is scheduling planting times in vegetable cultivation. For this purpose, determining the most suitable environmental conditions using growth, development, and yield models and using planned planting times is important. For example, the following model was developed to reveal the total dry matter production time in vegetables grown with adequate water and nutrients (Greenwood et al., 1976).

$w + K_1 \ln w + W_0 = K_2\, t$

w: Plant dry weight in tons per hectare

t: Number of days after emergence or after planting (growing time)

K_2 and K_1 constant coefficients depend on plant species and varieties

W_0: $w_0 + K_1 \ln w_0$ ("w_0" is the value of "w" at the beginning of the growing season)

't' is calculated as follows:

$$t = \frac{w + K_1 lnw + W0}{K_2}$$

For “t” to be high in the formula, 'W_0' and 'w' should also be high. A high W_0 value indicates that the seed and/or seedling are of good quality. In other words, the total root, stem and leaf dry weight are at the desired level and proportions when the seedling is planted. Quality seedlings affect plant growth time quite a lot. The K_2 value is similar for many plant species. A study concluded that the growth time curves of many vegetable species could be expressed with the same equation. The differences between these curves were mainly due to dry matter content (weight) and plant greening time (Greenwood et al., 1976). These variations were modeled mathematically and predicted the seedling production periods. Thus, it is aimed to make planned production under controlled greenhouse conditions by knowing the growth times of the plants in advance (Sarıbaş et al., 2017).

Today, where the demand for food increases with the increasing world population, the modeling of agricultural studies has gained importance for countries that produce large amounts of vegetables. By using multiple regression equations with the help of mathematical models, it is possible to predict the variation recorded in the vegetative growth of vegetables with temperature and light intensity (Kandemir and Uzun, 2019). With these models, vegetable production planning can be created especially in controlled greenhouses. Considering the effects of environmental factors (humidity, soil temperature, etc.) and cultural practices in addition to light and temperature, existing models can be developed, and it will be possible to use more planned vegetable growing systems by controlling plant growth in climate-controlled greenhouses.

4. CONCLUSION

The distribution of dry matter is closely related to plant growth and development. The cultural practices required to provide more dry matter accumulation in the parts to be harvested should also be done appropriately. One of the important effects of plant yield is that the part of the plant we grow that we want to harvest remains in the plant for a long time (Charles et al., 1986). Especially in vegetables, seed sowing and production elements such as seedlings, onions, tubers and planting times should be made considering the climatic conditions of each region. In this way, it is necessary to reveal the conditions under which dry matter will be more in the consumed parts of the plants grown. The reactions of vegetables to be grown under controlled conditions to light and temperature should also be revealed (Uzun, 2000).

In this sense, the relationship of vegetable species and varieties with environmental conditions in different regions of Türkiye should be investigated in detail. Conditions that will keep the net assimilation of any vegetable species or variety high in any region but will increase the yield and the quality of vegetables by reaching the maximum net assimilation slowly and steadily should be determined.

In practical terms, the growing season for vegetables is usually limited by adverse weather conditions. Low soil temperatures in early spring may delay the growing season in warm temperate climates. In this respect, frost-sensitive species should not be planted until the danger of spring frosts has passed, and they should also be harvested before the first frost of autumn. In markedly dry seasons and climates, growth support ends when the first rains in the growing season begin and soil moisture decreases. In most cases, the growing season duration is limited to the crop itself. Early harvesting is a practice that has several advantages for the growers, such as harvesting in good weather conditions and allowing the harvesting workload to spread over a longer period. Early ripened products can often be offered at a higher unit price.

Growing another winter crop or a fast-growing crop like a turnip does not allow the rest of the growing season to be wasted.

On the other hand, by extending the growing season, the yield can be increased and spread over a long period. With the use of frost-sensitive varieties, an application that will affect the growth period and yield will be realized. The growing season can be extended by adequate irrigation where water is limited. With the protection of some plants in glass greenhouses, the growing period can be extended from late winter to spring. As a result, the late-ripening cultivars generally have a low yield, but high yields will be obtained by spreading the yield over the total growing period. In early ripening varieties, the growing period is short, and the harvest is early, but a lower yield is obtained (Forbes et al., 1992).

REFERENCES

Acock, B., Charles Edwards, D.A., Fitter, D.J., Hand, D.W., Ludwig, L.J., Wilson Warren, J., Withers, A.C. (1978). The contribution of leaves from different levels within a tomato crop to canopy net photosynthesis: An experimental examination of two canopy models. *Journal of Experimental Botany* 29(4): 815-827.

Aktaş, H., Öztekin, G.B. (2019). Serada Biber Tarımı, Tarım Gündem Dergisi Özel Yayını, Nobel Yayıncılık, İzmir.

Al-Omran, A.M., Louki, I.I. (2011). Yield response of cucumber to deficit irrigation in greenhouses. *WIT Transactions on Ecology and the Environment* 145: 517-524.

Anonim (2022). Growing Vegetable Plants. http://osufacts.okstate.edu (Date of acces: 24.07.2022).

Aybak, H.Ç. (2002). Biber Yetiştiriciliği. Hasad Yayıncılık, İstanbul.

Balkaya, A. (2004). Modelling the effect of temperature on the germination speed in some legume crops. *Journal of Agronomy* 3(3): 179-183.

Balkaya, A., Kandemir, D. Sarıbaş, Ş. (2015). Türkiye sebze fidesi üretimindeki son gelişmeler. *TÜRKTOB Türkiye Tohumcular Birliği Dergisi* 13: 4-8.

Bleasdale, J.K.A. (1966). Plant growth and crop yield: The fourth Barnes Memorial Lecture. *Annals of Applied Biology 57*(2): 173-182.

Brewster, J.L. (1994). Onions and Other Vegetable Alliums. University Press, Cambridge.

Charles-Edwards, A.D., Doley, D., Rimmington, G.M. (1986). Modelling Plant Growth and Development. Academic Press.

Decoteau, D. (2000). Vegetable Crops, Prentice Hall, Upper Saddle River, New Jersey.

Demir, H. (2007). Vegetable seedling, problems and solutions in our country. *Hasad Bitkisel Üretim* 263: 68-74.

Demir, K., Çakırer, G. (2015). Kaliteli fide üretimini etkileyen faktörler. *TÜRKTOB-Türkiye Tohumcular Birliği Dergisi* 13: 12-15.

Demirsoy, M., Balkaya, A., Uzun, S. (2016). Farklı ışık kaynağı ve renk uygulamalarının patlıcan (*Solanum melongena* L.) fidelerinin büyüme parametreleri üzerine etkileri. *Selçuk Tarım Bilimleri Dergisi* 3(2): 238-247.

Ellis, R.H., Hadley, P., Roberts, E.H., Summerfield, R.J. (1990). Quantitative relationship between temperature and crop development and growth. Belhaven Press, New York, USA.

Forbes, J.C., Watson R.D. (1992). Plants in Agriculture. University Press, Cambridge.

Fordham R., Biggs A.G. (1985). Principles of vegetable crop production. Collins Profesional and Technical Books, London.

Ganesan, M., Subbiah Vijay, R. (2004). A case study on increasing tomato productivity in a low cost naturally ventilated greenhouse with different spacing. *Biodiversity Resources Management and Sustainable Use* 119-122.

Gaunt, R.E. (1995). The relationship between plant disease severity and yield. *Annual Review of Phytopathology* 33: 119-144.

Geboloğlu, N., Durukan, A., Sağlam, N., Doksöz, S., Şahin, S., Yılmaz, E. (2015). Patlıcanda fide gelişimi ve fide kalitesi ile paclobutrazol uygulamaları arasındaki ilişkiler. *Tarım Bilimleri Araştırma Dergisi* 8(1): 62-66.

Greenwood, D.J., Cleaver, T.J., Loquens, S.M.H., Nıendorf, K.B. (1977). Relationship between plant weight and growing period for vegetable crops in the United Kingdom. *Annals of Botany* 41(5): 987-997.

Hay, R.K.M., Walker, A.J. (1989). An Introduction to the physiology of crop yield. Longman Group UK Limited, United Kingdom.

Heuvelink, E., (1989). Influence of day and night temperature on the growth of young tomato plants. *Scientia Horticulturae* 38: 11-22.

Kandemir, D., Özer, H., Özkaraman, F., Uzun, S. (2013). The effect of different seed sowing media on the quality of cucumber seedlings. *The European Journal of Plant Science and Biotechnology*, 7(Special Issue 1): 66–69.

Kandemir, D., Uzun, S. (2019). Farklı ışık ve sıcaklık şartlarının sera biber yetiştiriciliğinde büyüme parametreleri üzerine kantitatif etkilerinin modellenmesi. *Anadolu Tarım Bilimleri Dergisi* 34(1): 1-11.

Kaur, A., Singh, S.P. (2013). Relationship between crop growth parameters and yield in brinjal as influenced by micrometeorological parameters. *Crop Improvement* 40(1): 65-68.

Khan, A., Jan, I.U., Ali, M., Jahangir, M.M., Karim, W., Khan, A.A., Ullah, M., Rafique, M.Z. (2021). Effect of different plant spacing on the performance of radish in the agro-climatic conditions of Swabi. *Pure and Applied Biology (PAB)* 5(4): 1120-1125.

Khan, M.A.I., Ueno, K., Horimoto, S., Komai, F., Tanaka, K., Ono, Y. (2007). Evaluation of the use of rice bran compost for eco-friendly weed control in organic farming, *American Journal of Environmental Sciences* 3(4): 234-239.

Koçer, G., Eltez, S. (2004). Serada domates yetiştiriciliğinde farklı renkte malç kullanımının verim, kalite ve sera beyaz sineği *Trialeurodes vaporariorum* (Westw.)Homoptera:Aleyrodidae) nimf populasyonuna olan etkileri üzerine araştırmalar. *Alatarım* 3(2): 36-42.

Kurtar, E.S., Odabaş, M.S. (2010). Modelling the yield of cucumber (*Cucumis sativus* L.) using light intensity, temperature and SPAD value. *Advances in Food Sciences* 32(3): 170-174.

Kürklü, A. (1998). Effects of temperature and time of harvest on the growth and yield of aubergine (*Solanum melongena* L.). *Turkish Journal of Agriculture and Forestry 22*(4): 341-348.

Mishra, P., Kumar, S., Yadav, S., Anushruti, Shukla, U. (2022). Effect of different varieties and spacings on growth and yield attributes of cabbage (*Brassica oleracea* var. capitata L.) under Lucknow conditions. *The Pharma Innovation Journal* 11(3): 1174-1178.

Odabaş, M., Gülümser, A., Uzun, S. (2007). The quantitative effects of temperature and light on growth, development and yield of faba bean (*Vicia faba* L.): I. growth. *International Journal of Agricultural Research* 2(9): 765-775.

Özdemir, A., Özer, H. (2015). Organik olarak yetiştirilen salkım domatesin (*Solanum lycopersicum* L.) verim ve kalitesi üzerine yaprak budamasının etkisi. *Anadolu Tarım Bilimleri Dergisi* 30(1): 1-6.

Özer, H. (2012). *Organik domates (Solanum lycopersium L.) yetiştiriçiliginde değişik masura, malç tipi ve organik gübrelerin büyüme, gelişme, verim ve kalite üzerine etkileri* (Doktora Tezi), Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun.

Özkaraman F. (2004). *Sera koşullarında sıcaklık, ışık ve farklı budamaların kavunda (Cucumis melo L.) büyüme, gelişme ve verime kantitatif etkileri* (Doktora Tezi), Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun.

Pamuk, G., Özgürel, M. (2005). Bitki büyüme modelleri: CERES-Maize örneği. *Ege Üniversitesi Ziraat Fakültesi Dergisi*, 42(1): 107-118.

Parkash, V., Singh, S., Deb, S.K., Ritchie, G.L., Wallace, R. W. (2021). Effect of deficit irrigation on physiology, plant growth, and fruit yield of cucumber cultivars. *Plant Stress* 1: 1-10.

Radics, L., Bognar, E.S., Bertschinger, L., Anderson, J.D. (2004). Comparison of different mulching methods for weed control in organic green bean and tomato. *Acta Horticulturae* 638: 189-196.

Saka A.K. (2012). *Serada ilk turfanda organik domates (Solanum lycopersicum L.) ve hıyar (Cucumis sativus L.) yetiştiriciliğinde farklı dikim sistemleri ve mesafelerinin büyüme, gelişme, verim ve kaliteye etkileri* (Yüksek Lisans Tezi), Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun.

Sandhu, R.K., Boyd, N.S., Zotarelli, L., Agehara, S., Peres, N. (2021). Effect of planting density on the yield and growth of intercropped tomatoes and peppers in Florida. *HortScience* 56(2): 286-290.

Sarıbaş, H.Ş. (2013). *Organik domates ve patlıcan fidesi üretiminde fide kalitesi ile çevre şartları arasındaki ilişkilerin belirlenmesi ve üretimin planlanması* (Yüksek Lisans Tezi), Ondokuz Mayıs Üniversitesi Fen Bilimleri Enstitüsü, Samsun.

Sarıbaş, H.Ş., Saka, A.K., Özer, H. (2018). Mathematical growth model for organically grown pepper transplants. *Biological Agriculture & Horticulture* 34(1): 10-17.

Seidel, S.J., Werisch, S., Schütze, N., Laber, H. (2017). Impact of irrigation on plant growth and development of white cabbage. *Agricultural Water Management* 187: 99-111.

Şaylan, L., Durak, M., Çaldağ, B. (1998). Dünya'da ve Türkiye'de bitki iklim (bitki gelişimi simulasyon) modelleri. *Tarım ve Orman Meteorolojisi Sempozyomu*. 21-23 Ekim, P.275-283, İstanbul, Türkiye.

Taiz, L., Zeiger, E. (2008). Bitki Fizyolojisi. PalmeYayıncılık, Ankara.

Tsuji, G., Uehara, G., Balas, S. (1994). DSSAT version 3. University of Hawaii, Honolulu, Hawaii.

Tüzel, Y., Gül, A., Daşgan, H.Y., Öztekin, G.B., Engindemiz S, Boyacı HF. (2015). Örtüaltı yetiştiriciliğinde değişimler ve yeni arayışlar. *Türkiye Ziraat Mühendisliği VIII. Teknik kongresi*. 12–16 Ocak, P. 685–709. Ankara, Türkiye.

Uçan, U., Uğur, A. (2021). Acceleration of growth in tomato seedlings grown with growth retardant. *Turkish Journal of Agriculture and Forestry* 45(5): 669-679.

Uğur, A., Kavak, S. (2007). The effects of PP 333 and CCC on seed germination and seedling height control of tomato. *Acta Horticulturae* 729: 205-208.

Uzun, S. (1996). *The quantitative effects of temperature and light environment on the growth, development and yield of tomato and aubergine* (PhD Thesis), The University of Reading, England.

Uzun, S. (1997). Sıcaklık ve işığın bitki büyüme, gelişme ve verimine etkisi (I.Büyüme). *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi* 12(1): 147-156.

Uzun, S. (2000). Sıcaklık ve ışığın bitki büyüme, gelişme ve verimine etkisi (III. Verim). *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi* 15(1): 105-108.

Uzun, S. (2001). Serada domates ve patlıcan yetiştiriciliğinde bazı büyüme ve verim parametreleri ile sıcaklık ve ışık arasındaki ilişkiler. *6. Ulusal Seracılık Sempozyumu*. 5-7 Eylül, P.97-102. Muğla, Türkiye.

Uzun, S. (2006). The quantitative effects of temperature and light on the number of leaves preceding the first fruiting inflorescence on the stem of tomato

(*Lycopersicon esculentum*, Mill.) and aubergine (*Solanum melongena* L.). *Scientia Horticulturae* 109(2): 142-146.

Uzun, S. (2007). Effect of light and temperature on the phenology and maturation of the fruit of eggplant (*Solanum melongena*) grown in greenhouses. *New Zealand Journal of Crop and Horticultural Science* 35(1): 51-59.

Uzun, S. Marangoz, D., Özkaraman, F. (2001). Modeling the time elapsing from seed sowing to emergence in some vegetable crops. *Pakistan Journal of Biological Sciences* 4(4): 442-445.

Uzun, S., Demir, Y. (1996). Sıcaklık ve ışığın bitki büyüme, gelişme ve verimine etkisi (II.Gelişme). *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi* 11(3): 201-212.

Uzun, S., Demir, Y., Özkaraman, F. (1998). Bitkilerde ışık kesimi ve kuru madde üretimi. *Ondokuz Mayıs Üniversitesi Ziraat Fakültesi Dergisi* 13(2):133-154.

Went, F.W. (1945). Plant growth under controlled conditions, V. The relation between age, light, variety, and thermoperiodicity of tomatoes. *American Journal of Botany* (32): 469-479.

Yörük, E., Duran, H., Eren, E., Özer, H. (2021). Determination of growth models in organic lettuce cultivation. *Anadolu Tarım Bilimleri Dergisi* 36(2): 276-281.

Zanic, K., Ban, D., Ban, G.S., Culjak, G.T., Dumicic, K. (2009). Response of alate aphid species to mulch colour in watermelon. *Journal of Food, Agriculture & Environment* 7(3): 496-502.

CHAPTER 7

THE TRACEABILITY OF AGRICULTURAL SUPPLY CHAIN BY BLOCKCHAIN TECHNOLOGY

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1. INTRODUCTION

As Maslow (1943) stated, nutrition is one of the essential needs in the hierarchy of needs. Various risks may occur if the nutritional needs are not fulfilled healthily and safely. Many effects, such as the increase in the world population and the pollution of nature due to global industrial activities, trigger global climate change. Arable land in the world is decreasing. As a result of the developing global trade, there are a wide variety of agricultural food (AF) products in circulation between countries. These biological materials imported from distant countries may contain different levels of preservative chemicals, pesticides, and veterinary drug residues. It may even carry microbiological and especially zoonotic risks. Due to many global effects like this, the microbial ecology in our ecosystem is changing. Deteriorating or pathogenic microorganisms can acquire resistance. In addition to the health risks they carry, these elements also cause a loss of commercial and economic value. Due to these risks, food safety's importance is increasing daily. The agricultural food supply chain (AFSC) must be traceable at every point, from cultivation, breeding, processing, distribution, preparation, and consumption, from farm to fork or field to table. Also, the factors such as the increase in population and technological opportunities, infrastructure developments, and the increase in the level of consciousness of consumers have made food safety more critical. While aiming for sustainable development, AFSCs face some important environmental, economic, and social challenges. As stated in studies (FAO, 2016; Nemarumane and Mbohwa, 2013), especially in the social field in developing countries, some issues related to gender equality, pay, and health pose a more severe challenge. Also, according to Matopoulos et al. (2007), public awareness of purchasing healthy and environmentally friendly products is increasing. To improve the performance of supply chains and to gain a collective competitive advantage, AF systems must collaborate among heterogeneous stakeholders. Thanks to this collaborative system, stakeholders also have the opportunity to increase their market share and increase their profit margins (Fearne et al., 2001). Furthermore, through Cooperation, conflicts between stakeholders can be reduced. Each stakeholder can be encouraged to raise their responsibility for sustainability (Pomeroy et al., 2007). Hamprecht et al. (2005) stated that an effective communication method improves environmental, social, and economic standards among stakeholders (farmers and traders).

2. TRACEABILITY

Traceability is an applicable term to many industrial products; also, in the food industry it is widely used. ISO 22005 (2007) defines traceability as "the ability to trace the history, application or location of an entity, using recorded identifications." Further, ISO guidelines specify that traceability may refer to the origin of materials and parts, the processing history, and the distribution and location of the product after delivery. ISO 22005:2007 gives the principles of basic specific requirements for designing and implementing a feed and food traceability structure. They were applied by an organization operating at any feed and food chain step. It is intended to be flexible enough to allow feed and food organizations to achieve identified objectives. The traceability system is a technical tool to manage an organization conforming to its defined objectives. It is applicable when necessary to determine a product's history, location, or proper components. The European Union (EU) provisions, for example, 178/2002 (EU, 2002), narrowed this definition further and addressed it for the food industry. Traceability has been expressed as "the ability to track and trace a food, feed, food-producing animal or substance that is intended or expected." All production, processing, and distribution stages are incorporated into food or feed. The Codex Alimentarius Commission (CAC, 2005) defines traceability as "the ability to track the movement of a feed or food through specified stages of production, processing, and distribution." ISO 22005 (2007) follows the definition made by the Codex commission. It defines a system design on traceability in food and feed and what general principles and basic requirements are needed for the designed system to be applicable. Olsen and Borit's (2013) definition of traceability is "the ability to access any or all information relating to that which is under consideration, throughout its entire life cycle, using recorded identifications." The Law "Veterinary Services Plant Health Food and Feed Law" of the Republic of Turkey No. 5996 (TC, 2010), traceability is defined as "the ability to trace and trace a substance intended or expected to be found in plant products, food, and feed, the animal or plant from which the food is obtained, throughout all stages of production, processing, and distribution." In recent years, traceability has become recognized as an essential tool for guaranteeing food safety and quality. On the other hand, the design of a traceability system requires a thorough rethinking and reorganizing of the whole AFSC. Verdouw et al. (2013) stated that in the years when the study was conducted, the central information systems in the AFSCs did not provide the requirements such as transparency, traceability, and auditability; they were only contented with tracking and storing orders and deliveries. Consumers' demand for these requirements that increase food safety and quality is increasing.

3. BLOCKCHAIN TECHNOLOGY

It can do this without being dependent on any central third-party intermediaries. A potential solution to mitigate such problems and concerns is to use blockchain technology (BCT), a peer-to-peer digital ledger without any reliance on centralized servers. All records are stored on a blockchain; this distributed ledger is by design immutable, consisting of hashes based on a given general agreement and reached at least by the majority of the network's peers. Thus, they provide an auditable and transparent source of information.

Distributed ledger technology is a data storage technology in which the data is kept distributed among the participants, the specified verification algorithm does the data recording in the ledger, and any participant cannot change the data. In traditional approaches, data is kept on servers controlled by a central participant. Although this method is helpful initially, it gradually loses its functionality with technological developments. Keeping data on one or more devices and changing it by authorized users causes it to become an open target for cyber attacks. In addition, with the developments in the Internet of Things (IoT) technology and the increased number of devices connected, central servers cannot respond to this processing density. In order to eliminate all these problems, distributed ledger technology has emerged, and studies are being carried out on it. Some of these are Blockchain, Hashgraph, Tangle, Holochain, and Tempo distributed ledger technologies. A simple AFSC management process with involved actors is introduced in the following, depicted in Fig. 1 (Verdouw et al., 2013):

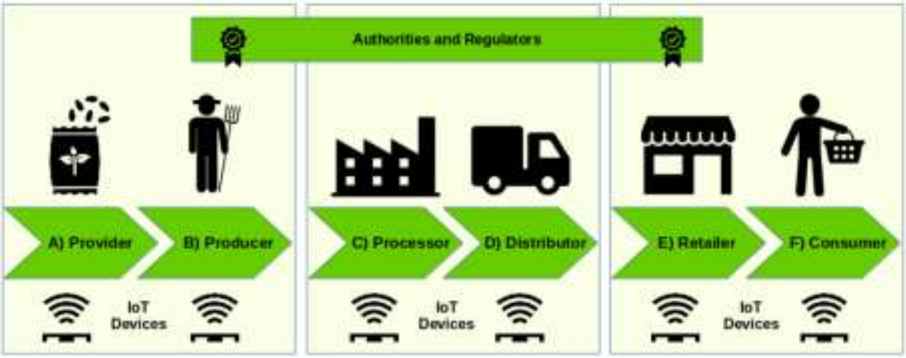

Figure 1. A simple version of the management of AFSC (Verdouw et al., 2013).

"A) **provider:** providers of raw materials, such as seeds and nutrients, but also pesticides, chemicals, etc.;

B) **producer:** usually the farmer, e.g., the responsible for the actions from seeding/planting to harvesting;

C) **processor**: this actor may perform various actions, from simple packaging to more complex processes (e.g., pressing of the olives);

D) **distributor:** this actor is responsible for moving the output of the processor (e.g., the product) from the processor's site to retailers;

E) **retailer:** this actor is responsible for selling the products, representing either small local stores or big supermarkets;

F) **consumer**: the final element of the chain."

Related actors must comply with the authorities' stipulated legislation, standards, guides, and policies throughout the process. Moreover, from another aspect, sensor networks of blockchain-situated traceability opinion would require a stable link to their care-located peer instead of necessary connectivity to a central cloud. Thus, blockchain components expose all properties needed for decentralizing AF traceability networks. At the same time, elements make traceable data of every available supply chain. The supply chain and hierarchical structure of potential benefits are given in Fig. 2 (Mukherjee et al., 2022).

To assist sustainable applications and ease coordination, cooperation, and traceability along the supply chain, companies in the AF sector need to develop their supply chain capabilities. Integrating BCT with the traditional supply chain will facilitate solving existing supply chain challenges and mitigating risks. Thus, it will be possible to ensure sustainability within the AF industry (Kshetri, 2018; Nandi et al., 2020).

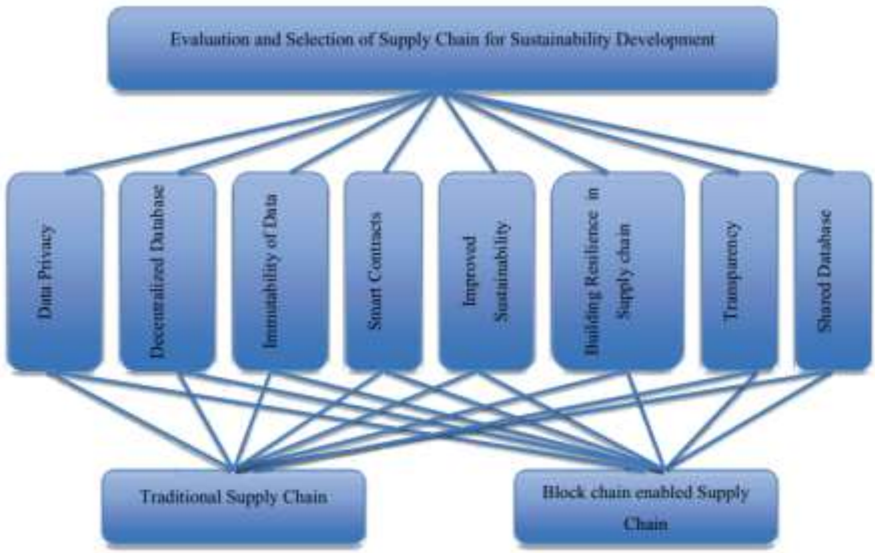

Figure 2. The supply chain hierarchical structure selection for the development of sustainability (Mukherjee et al., 2022)

However, transitioning from a traditional supply chain to a blockchain-enabled supply chain requires significant economic commitments. Moreover, It also pressures companies to change their existing AFSCs for scalability to meet their sustainability needs (Nayak et al., 2019). Thus, in food processing along the whole AFSC, several Research and Development communities are focusing on adopting to enable remote monitoring of the conditions of some specific IoT technologies, such as Wireless or Wire Sensor Networks, Radio Frequency Identification (RFID)s, and or Diurnal-Cheaper-Connected devices. Mainly within the financial technology (Fin-Tech) industry, activities around BCT have witnessed a blowup in development and research subjects for the last few years. Blockchain-enabled AFSC administration requires a lot of renovation and technology knowledge. The preliminary stages of blockchain appropriation faced several challenges that required technical instructions to discuss the technology's benefits (Yadav and Singh, 2020; Janssen et al., 2020). Therefore, companies must ensure that the investments in BCT are justified in bringing sustainability or sustainability-concerned benefits from this technology. In other words, whether this attribute is significantly relevant to the benefit of the traditional food-supply chain needs to be analyzed. However, using the BCT and IoT techniques in the AF area is still an under-discover, yet worth-to-explore, research area.

4. BCT APPLICATIONS IN SUSTAINABLE AFSC

Global food markets' high competition and great consumer expectations, AF companies have pushed to fund modern technologies of the AFSC (Yadav et al., 2019; Kim et al., 2019). Over the years, modern technologies such as the IoT, cloud computing, and BCT have been used to demand changes and manage supply, food safety subjects, and sustainability factors (Verdouw et al., 2015; Carbonell, 2016; Bronson and Knezevic, 2016; Wang and Yue, 2017). This trend has awakened interest in industry and academia (Schmidt and Wagner, 2019; Dolgui et al., 2020; Chang et al., 2020; Koh et al., 2020; Pournader et al., 2020).

Maru et al. (2018) indicated that blockchain is applicable in various fields such as soil-management, soil-use, registry keeping, purchase details, use of farming equipment, pesticides, traceability system, and financial transactions in the AFSC. As this technology has bounded to circular finance, it can also develop the sustainability factor in the AFSC (Mac Arthur et al., 2015; Jabbour et al., 2017; Kalmykova et al., 2018; Jabbour et al., 2019). Blockchain technology; performs storage trouble-free, decreases product fraud and adulteration, and improves security in the AFSC, critical areas of concern for the consumer and government. It brings to life the farm-to-fork concept by providing real-time data tracking of farm products (Ge et al., 2017). BCT offers transparency in the business's value chain, helps to make data-based decisions, and supports reliable data clusters and transactions in real time. (Xiong et al., 2010). Through smart contracts used for these transactions, misapplication and fraud can be reduced (Sylvester, 2019). These benefits support sustainability in the AFSC (Li and Wang, 2018; Tian, 2016).

Eliminating BCT intermediaries contributes to sustainability by providing equal opportunities for small stakeholders. In this way, BCT allows farmers to decide which crop to grow at what prices in a given season. In this way, it encourages farming to be more rational by making it resistant to price vulnerabilities. Thanks to this technology, consumers know the farmers who grow seasonal, cheap, and local crops very well.

BCT; build digital trust between consumers and manufacturers. Thus, it also brings proactivity in minimizing on-chain transaction costs (Yiannas, 2018). Blockchain is a farm-to-fork technology. It covers the entire farmer, producer, and distributor network. It offers real-time traceability of products from the starting point to the final destination. Thus, it performs a reliable flow of information and transactions between stakeholders. BCT provides a complete guarantee of food safety, food quality, and seasonality in the AF supply. This makes the supply chain a flexible system that covers financial, technical, and societal aspects using decentralized solutions on the BCT. This flexible factor further increases the relationship coefficient, rapid trust, and cooperation levels between stakeholders (Dubey et al., 2019).

The application of the BCT structure is shown in Figure 3. BCT has been identified to have considerable direct implications for ‘Trust, Transparency, and Traceability' shown with the biggest arrow in the figure. BCT could strengthen supply-chain members and fair trade by directly impacting sustainable AFSCs. Successful implementation of BCT can potentially increase profitability and efficiency. It is seen in the other pillars, the impact of the less intense effect of BCT indirectly by its strengths in delivering immutable, decentralized, and transparent information. Resources can be qualified more, while recalls, fraud, and food waste can be prevented. Furthermore, communicating reliable information In the AF area, the stored records must be tamper-proof to keep trust and reliability along the supply chain. As given in Figure 3, BCT offers consumers an auditable and transparent information source, increases their knowledge, and promotes sustainable and healthy investment in decisions. (Wünsche and Fernqvist, 2022).

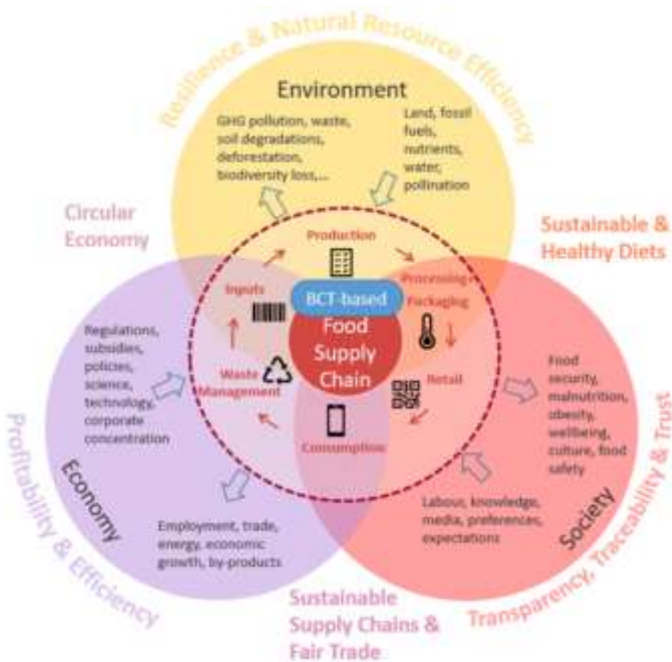

Figure 3. The framework of the sustainable food system and potential influences from BCT (BCT) (IFST 2022)

Table 1. The usage of BCT and challenges for the food sector (Wünsche and Fernqvist, 2022)

Food System Framework Category	Importance in Food System Transformation	Use of BCT
Resilience and resource efficiency	Food systems contribute substantially to climate change Highly reliant on functioning ecosystems Food insecurity as consequence of climate change	Store and share data from agricultural precision technologies Prevent waste through individualised perishability dates More efficient planning via increased transparency
Sustainable & healthy diets	Plant-based diets are more environmentally friendly Consumer education and preferences are crucial for rethinking the food system Globally higher demand for energy-intensive foods Environmental awareness in society is increasing Higher demand for sustainable products	Provide the consumer with transparent and verified data for better decision making
Circular economy	Food waste minimisation Nutrient recycling	Better planning can improve recycling Motivation via crypto tokens Traceability helps to identify flaws in the supply chain and prevent food contamination and spoilage
Profitability and efficiency	Food waste and GHG emissions are inefficiencies in food systems Lack of digitalisation Long and complex supply chains Opacity/Transparency	Reduced costs (via disintermediation) increase economic sustainability Minimisation of human error Safe data transfer reduces risk Disintermediation of supply chains
Sustainable supply chains and fair trade	Concentration of power to a few multinational corporations Absence of cooperation and policies on international level	Immutability and reliability of data can detect and prevent corruption and increase trust in the supply chain BCT reduces information asymmetry and improves equality in bargaining power Collusion can be monitored and prevented Verification of ethical working conditions and fair trade
Transparency, traceability, and trust	Distance between producers and consumers Environmental impacts of food are rarely visible to the consumer Lack of trust in brands and labels Food safety concerns	BCT increases transparency for all supply chain members Transparency increases trust, brand image and decision-making

The current food sector faces many sustainability challenges, as pointed out by the International Panel on Climate Change (IPCC) and AF Security (FAO) (Wünsche and Fernqvist, 2022). The use of BCT and its relationship with essential sustainability issues in food-sector transformation is provided in Table 1.

Amentae et al. (2021) examined 76 thematic articles in the Scopus database index. These articles were about traceability, sustainability, and other performances, including productivity, safety, resilience, and risk management during crises. The study aimed to review the literature published between 2017 and 2021) and examined the potential contribution of digitalization of the AF systems to such a transition. In the digitalization of the AF systems, identifying digital technologies' present possibilities and challenges in the transition toward a sustainable food system. The specific subjects of the study were to identify the main concluding remarks of the traceability, sustainability, and other performances, including productivity, safety, risk management, and resilience during crises were summarized respectively in Table 1, 2, and 3.

As indicated in the tables at the end of the study, Amentae et al. (2021) noted that the arrangement of outcome descriptions by central application area in Tables 2 to 4 highlights how digitalization serves different key performance areas of the food system. The problems obtained from the articles were divided into categories in this study. Traceability is summarized in Table 2, sustainability in Table 3, and other issues related to AFSC management in food loss and reducing food waste are summarized in Table 4. As seen from Table 3, the reviewed literature showed that AF system digitization had contributed to achieving sustainability goals across AFSCs. Concluding remarks from the reviewed articles revealed that digital technologies in food systems help achieve social, economic, and environmental sustainability goals. The results in Table 3 show that using digital technologies throughout the food chain enables the achievement of sustainability goals and overall food safety and security goals.

Table 4 summarizes the main results of the reviewed articles and presents them in a tabular form. The results in Table 4 show the digitization of the system throughout the AFSCs; It demonstrates that it helps increase productivity, product quality, and food safety and supports new business models. Also, the review in Table 4 shows that using digital technologies allows for risk-taking and opportunism. It has been demonstrated that it enables the system to remain resilient during health crises such as the COVID-

19 epidemic, reducing costs along food chains and increasing competitiveness.

Table 2. Traceability (Amentae et al., 2021)

- Smart contracts using end-to-end blockchain-based agri-food supply chain solutions are efficient and robust in terms of traceability, trading, delivery, and reputation.
- A blockchain-based framework combined with deep reinforcement learning (DRL) in food supply chain management (FSCM) provides reliable product traceability, higher profitability, and supply chain flexibility.
- For small and medium enterprises (SMEs) in the Malaysian halal agro-food chain, technological advances such as IoThave high potential for achieving product traceability.
- Traceability, price, trust, compliance, coordination, and control (in that order of relative importance) influence adoption of BCT.
- A model for agro-food traceability can be developed based on comprehensive and quantifiable granularity concepts.
- Continuous traceability between storage and logistics improves the breadth, depth, and precision.
- A blockchain-based system guarantees food quality and process safety traceability.
- Blockchain & IoT empower a transparent, auditable, and traceable food system.
- BCT as the start of revolutionary food supply chain tools will allow consumers to really know where their food comes from (traceability).
- Blockchain can help improve food traceability, information transparency, and recall efficiency.
- Blockchain enhance FSCM and traceability in food distribution.
- Asymmetries between traceability systems along the supply chain affect inventory and food quality.
- Blockchain implementation improves traceability in the food system.
- Use of BCT in agri-food supply chains provides trusted track, trace, and provenance information to the focal firm and consumers that leverages efforts for sustainability.
- BCT can be considered a true innovation and relevant approach to assure traceability and authenticity in the food supply chain, data acquisition and management.
- AgriBlockIoT (integrated IoT and blockchain technologies) enables creation of transparent, fault-tolerant, immutable,and auditable agri-food traceability systems.
- Digitalization considering blockchain effects is essential for sustainable agro-supply chain management (ASCM), due to the ease in product traceability, security, and transactions.
- Blockchain supports non-tampering data, thus increasing consumer confidence in FSCM and food products; integration of blockchain with big-data analytics alleviates the slight data speed downside.
- Blockchain overcomes privacy and security challenges through smart contracts, monitoring counterfeiting, and traceability systems to ensure food safety and security.
- In food supply chains, increased transparency, traceability, and trust are direct attributes of blockchain-based technologies, while sustainability improvements are an indirect attribute.
- Regardless of the challenges faced, introduction of new technologies such as IoT to the food chain has potential to make food production a truly transparent process.
- A framework for reliable, auditable, and trackable FSCM ensures transaction integrity, immutability, and transparency of perishable products.
- The decentralized E-supply chain model performs well in fresh produce supply chains from the perspective of quality protection through traceable information.
- Implementation of big-data analytics, cloud computing, and IoT can transform and upgrade FSCM to a smart future and more sustainable and adaptive food supply chains.
- Data-driven agri-food supply chains (AFSCs) are a means for sustainability of the food system.

Table 3. Sustainability (Amentae et al., 2021)

- Machine learning (ML) techniques can benefit agriculture supply chains and lead to sustainability.
- Geographic information system (GIS)-based fuzzy applications can be used in land suitability assessment, real-time crop quality inspections; real-time weather forecasting, locations models for cold storage, and real-time perishable agri-transportation model considering socio-economic factors, among others.
- Food security, safety, and sustainability can be addressed through big data applications.
- BCT can contribute to a more sustainable food industry and help to achieve traceability by irreversibly and immutably storing data.
- To produce more food more sustainably, the use of digital technologies offers potential solutions.
- Smart technologies as tools can lead to customization of sustainable food supply chains.
- Blockchain enables sustainability performance of supply chains and promotes United Nation Sustainable Development Goals (SDGs).
- Information & communication technology (ICT) can contribute to agro-food sustainability transition.
- A data-driven analysis framework that considers multiple aspects of food supply chains can help move sustainable planning beyond the traceability objective.
- Frameworks of Industry 4.0 and models, algorithms, heuristics, and metaheuristics technologies can enable sustainability in food supply chains.
- Artificial intelligence (AI) and digital technologies can help create sustainable business models that increase productivity, reduce production costs and emissions, and improve correspondence in markets.
- Blockchain plays a vital role in food system sustainability.
- Cloud computing provides useful metadata for digital certifications using global positioning system (GPS) and other sensor technologies, and satisfies the objectives of the sustainable business model by reducing transaction costs and strengthening alliances between stakeholders.
- GIS-based analysis integrated with current data streaming and blockchain platforms enhances trust and sustainable integration of food supply chains.
- A framework driven by AI technologies can contribute to sustainable financing stream for food and drink supply chains.
- BCT has the potential to improve supply chain sustainability in the areas of environmental protection, social equity, and governance efficiency indicators.
- In some regions, the current adoption of digital agriculture appears to be driven more by economic sustainability than by social or environmental sustainability, so efforts by policymakers and by social or environmental sustainability, so efforts by policymakers and stakeholders are needed.
- Digitalization and use of smart technologies (IoT, robots, AI, blockchain) in agriculture enable transition of the sector to sustainability in all its three forms: economic, social, and environmental.

Table 4. The other functions including safety, productivity, risk management, and resilience during crises (Amentae et al., 2021)

- A blockchain-based framework (Food Safety Quick Response Block, FoodSQRBlock) using QR codes can be successful.
- Food safety, product quality, and associated economic benefits in the dairy industry can be achieved through technological innovation and introducing FSCM practices into lean and green initiatives.
- Blockchain, in the context of e-agriculture, has potential for reshaping the entire sector and helping to resolve the food crisis.
- Sensor-based mobile application technologies for monitoring tracking information and storage conditions of agri-food products are useful in real-operation contexts.
- Wireless sensor networks, cloud computing, IoT, image processing, convolutional neural networks, and remote sensing can improve coffee supply chains.
- Digitalization has disrupted food distributor models; E-commerce models and IoT are essential factors causing retailers to innovate their business models.
- Blockchain-based ASCM can help restrict opportunism in agri-food chains by facilitating management of uncertainty and asset specificity.
- Digital technologies alter agricultural supply chains and offer new ways to create value.
- Competitive advantages can be achieved using ICT solutions for widening and maintaining relations through more effective information flow with partners and consumers.
- Use of blockchain technologies in cooperatives has significant societal impacts in terms of improving milk quality, animal welfare, and milk safety, traceability and transparency.
- The agro-food sector is among the sectors positively impacted by blockchain.
- Technological integrations of blockchain and IoT provide solutions for data security and performance issues in precision agriculture.

4. CONCLUSION

A wide variety of global factors give the AF industry a severe stimulus to traceability and inspection of the sustainability of the system's inputs. The modern AFSC operates in a multi-layered ecosystem of stakeholders. It is a non-linear and non-sequential chain from the information supply to the shipping of final products to multiple geographic locations. AF businesses face daunting challenges in terms of low automation levels, lack of industrialization, poor management skills, inconsistent and fragmented information, adulteration, and food safety issues that ultimately render the supply chain inefficient.

Controlling traceability means managing the economic, social, and environmental performance of the AFSC. Implementing new digital technologies such as BCT offers many options for companies to expand their existing AFSC controls to cover these aspects efficiently. Since this distributed ledger technology brings a new philosophy, it will be beneficial to make necessary regulatory arrangements and increase social awareness and technical studies to catch up with technology's future development. BCT has emerged as a pragmatic solution to enable and integrate sustainability practices in AFSCs today. Companies in the AF sector face constant pressure to develop a sustainable supply chain in the competitive market. Therefore, adopting BCT could be a promising development for these industries.

REFERENCES

Amentae, T.K., Gebresenbet, G. (2021). Digitalization and future agro-food supply chain management: a literature-based implications. *Sustainability*, 13: 12181.

Bronson, K., Knezevic, I. (2016). Big data in food and agriculture. *Big Data & Society* 3(1):2053951716648174.

CAC (2005). Codex procedural manual, 15th edn.

Carbonell, I. (2016). The ethics of big data in big agriculture. Internet Policy Review, 5(1) Available at SSRN: https://ssrn.com/abstract=2772247.

Chang, Y., Lakovou, E., Shi, W. (2020). Blockchain in global supply chains and cross border trade: a critical synthesis of the state-of-the-art, challenges and opportunities. *Int J Prod Res* 58(7):2082–2099.

Dolgui, A., Ivanov, D., Portryasaev, S., Sokolov, B., Ivanov, M. (2020). Blockchain oriented dynamic modeling of smart contract design and execution in the supply chain. *Int J Prod Res* 58(7):2184–2199.

Dubey, R., Gunasekaran, A., Childe, S.J., Fosso Wamba, S., Roubaud, D., Foropon, C. (2019). Empirical investigation of data analytics capability and organizational flexibility as complements to supply chain resilience. *Int J Prod Res* 1–19.

EU (2002). Regulation (EC) No 178/2002 of the European Parliament and of the Council of 28 January 2002.

FAO (2016). Social Protection Division (ESP).

Fawcett, S.E., Osterhaus, P., Magnan, G.M., Brau, J.C., McCarter, M.W. (2007). Information sharing and supply chain performance: the role of connectivity and willingness. *Supply Chain Manag. Int. J.* 12: 358e368.

Fearne, A., Hughes, D., Duffy, R. (2001). Concepts of collaboration: supply chain management in a global food industry. In: Eastham, J.F., Sharples, L., Ball, S.D. (Eds.), Food Supply Chain Management. Butterworth-Heinemann, Oxford, 55e89.

Ge, L., Brewster, C., Spek, J., Smeen, A., Top, J., van Diepen, F., Klaase, B., Graumans, C., de Ruyter de Wildt, M. (2017). Blockchain for agriculture and food: Findings from the pilot study. (Wageningen Economic Research report; No. 2017-112). *Wageningen Economic Research*.

Hamprecht, J., Corsten, D., Noll, M., Meier, E. (2005). Controlling the sustainability of food supply chains. *Supply Chain Manag. Int. J.* 10, 7e10.

IFST (2022). Food System Framework: A Focus on Sustainability. https://www.ifst.org/our-resources/science-and-policy-resources/sustainable-food-system. Date of access: 22 October 2022.

ISO 22005 (2007). Global Food Standard. https://www.iso.org/standard/36297.html. Date of access: 02 November 2022.

Jabbour, C.J.C., de Sousa Jabbour A.B.L., Sarkis, J., Godinho Filho, M. (2017). Unlocking the circular economy through new business models based on large-scale data: An Integrative Framework and Research Agenda. *Technol Forecast Soc Chang* 144:546–552.

Jabbour, C.J.C., Sarkis, J., de Sousa Jabbour, A.B.L., Renwick, D.W.S., Singh, S.K., Grebinevych, O., Kruglianskas, I., Godinho Filho, M. (2019). Who is in charge? A review and a research agenda on the 'human side' of the circular economy. *J Clean Prod* 222:793–801.

Janssen, M., Weerakkody, V., Ismagilova, E., Sivarajah, U., Irani, Z. (2020). A framework for analyzing blockchain technology adoption: Integrating institutional market and technical factors. *Int J Inf Manag* 50:302-309.

Kalmykova, Y., Sadagopan, M., Rosado, L. (2018). Circular economy-from review of theories and practices to development of implementation tools. *Resour Conserv Recycl* 135:190–201.

Kim, HM, Laskowski, M. (2018). Toward an ontology-driven blockchain design for supply-chain provenance. *Intell Sys Acc Fin Mgmt* 25: 18– 27.

Kim, M., Hilton, B., Burks, Z., Reyes, J. (2019). Integrating blockchain, smart contract-tokens, and iot to design a food traceability solution. In: *Proceedings of the 9th IEEE Annual Information Technology, Electronics and Mobile Communication Conference*, Vancouver, Canada, 335–340.

Koh, L., Dolgui, A., Sarkis, J. (2020). Blockchain in transport and logistics-paradigms and transitions. *Int J Prod Res* 58(7):2054–2062.

Kshetri, N. (2017). Will blockchain emerge as a tool to break the poverty chain in the Global South? *Third World Quarterly* 38(8):1710–1732

Kshetri, N. (2018). Blockchain's roles in meeting key supply chain management objectives. *Int J Inf Manage* 39:80–89.

Li, J., Wang, X. (2018). Research on the application of blockchain in the traceability system of agricultural products. *2nd IEEE advanced information management, communicates, electronic and automation control conference* (IMCEC) 2637-2640.

MacArthur, E., Zumwinkel, K., Stuchtey, M. (2015). Growth within a circular economy vision for a competitive Europe. Ellen MacArthur Foundation.

Maru, A., Berne, D., Beer, J.D., Ballantyne, P.G., Pesce, V., Kalyesubula, S., Chavez, J. (2018). Digital and data-driven agriculture: Harnessing the power of data for smallholders. Available at: Global F Agricultural Research and Innovation https ://cgspa ce.cgiar.org/bitstream/handle/10568 /92477 /GFAR-GODAN - CTA-white - paper-final. pd.

Maslow, A.H. (1943). A theory of human motivation. *Psychological Review* 50(1):370–396.

Matopoulos, A., Doukidis, G.I., Vlachopoulou, M., Manthou, V., Manos, B. (2007). A conceptual framework for supply chain collaboration: empirical evidence from the agri-food industry. *Supply Chain Manag. Int* J. 12, 177e186.

Mukherjee, A., Singh, R.K., Mishra, R., Bug, S. (2022). Application of blockchain technology for sustainability development in agricultural supply chain: justification framework. *Oper Manag Res 15:* 46–61.

Nandi, M.L., Nandi, S., Moya, H., Kaynak, H. (2020). Blockchain technology-enabled supply chain systems and supply chain performance: a resource-based view. Supply Chain Management: *An International Journal* 25/6 841–862 Emerald Publishing Limited ISSN 1359–8546.

Nayak, G., Dhaigude, A.S., Yogesh, P.P. (2019). A conceptual model of sustainable supply chain management in small and medium enterprises using blockchain technology. *Cogent Economics & Finance* 7:1

Nemarumane, T.M., Mbohwa, C. (2013). Social impact assessment of sugar production operations in South Africa: a social life cycle assessment perspective. In: Nee, A., Song, B., Ong, SK. (eds) Re-engineering Manufacturing for Sustainability. Springer, Singapore.

Olsen, P., Borit, M. (2013). How to define traceability. *Trends Food Sci Technol* 29(2):142–150.

Pomeroy, R., Parks, J., Pollnac, R., Campson, T., Genio, E., Marlessy, C., Holle, E., Pido, M., Nissapa, A., Boromthanarat, S., Nguyen, T.H. (2007). Fish wars: Conflict and collaboration in fisheries management in Southeast Asia, *Marine Policy, Elsevier* 31(6): 645-656.

Pournader, M., Shi, Y., Seuring, S., Lenny, Koh. S.C. (2020). Blockchain applications in supply chains, transport and logistics: A systematic review of the literature. *Int J Prod Res* 58(7):2063–2081.

Pretty, J.N., Morison, J.I.L., Hine, R.E. (2003). Reducing food poverty by increasing agricultural sustainability in developing countries. *Agric. Ecosyst. Environ.* 95: 217e234.

Schmidt, C.G., Wagner, S.M. (2019). Blockchain and supply chain relations: a transaction cost theory perspective. *J Purch Supply Manag* 25(4).

Şafak, E., Arslan, Ç., Gözütok, M., Köprülü, T. (2021). Dağıtık defter teknolojileri ve uygulama alanları üzerine bir inceleme. *European Journal of Science and Technology* Special Issue 29: 36-45.

TC (2010). Law No: 5996 of Turkish Republic. Veterinary services plant health food and feed law official gazette No: 27610 13 June 2010 Date of access: 6/11/2010.

Tian, F. (2016). An agri-food supply chain traceability system for China based on RFID & blockchain technology. In: 2016 13th *International Conference on Service Systems and Service Management (ICSSSM)*. IEEE, 1-6.

UN (2015). Transforming our world: The 2030 agenda for sustainable development. United Nations, Department of Economic and Social Affairs, New York.

Verdouw, C.N., Beulens, A.J., Reijers, H.A., van der Vorst, J.G. (2015). A control model for object virtualization in supply chain management. *Comput Ind* 68:116–131.

Wang, J., Yue, H. (2017). Food safety pre-warning system based on data mining for a sustainable food supply chain. *Food Control* 73:223–229.

Verdouw, C., Sundmaeker, H., Meyer, F., Wolfert, J., Verhoosel, J. (2013). Smart agri-food logistics: requirements for the future internet in: Dynamics in Logistics. *Springer* 247–257.

Wünsche, J.F., Fernqvist, F. (2022). The potential of blockchain technology in the transition towards sustainable food systems. *Sustainability* 14, 7739.

Xiong, W., Holman, I., Lina, E., Conway, D., Jiang, J., Xu, Y., Li, Y. (2010). Climate change, water availability and future cereal production in China. *Agric Ecosyst Environ* 135(1–2):58–69.

Yadav, S., Singh, S.P. (2020). Blockchain critical success factors for sustainable supply chain. *Resources Conserv Recycl* 152:104505.

Yadav, V.S., Singh, A.R. (2019). Use of blockchain to solve select issues of Indian farmers. *AIP Conference Proceedings* 2148.

Yiannas, F. (2018). A new era of food transparency powered by blockchain. *Innov Technol Gov Glob* 12(1–2):46–56.

CHAPTER 8

AGRICULTURAL TERRACING

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1. INTRODUCTION

Terraces are one of the physical protection methods used to combat water erosion. Soil embankments established parallel level curves to prevent erosion by rainwater on sloping lands are called terraces. In places where erosion and flood damage cannot be prevented by plant rotation, tillage parallel to leveling curves, planting on strips, etc., the cultivated lands should be terraced (Figure 1).

Terrace is defined as a flattened section of a sloping or hilly area in farmland. With terracing, the flow rate of runoff water is reduced, and rainwater seeps into the lower layers of the soil and helps prevent erosion.

Terraces are one of the most important soil and water conservation measures to prevent erosion and water conservation on sloping lands, and terracing is a measure consisting of channels and ridges perpendicular to the slope (Taysun, 1989; Çalık, 2020).

Figure 1. Terracing (Anonymous, 2022a)

Terraces are structures created by digging along the contours of the slope on sloping lands and piling the resulting soil into the lower part. With terracing, the steepness of the slope is reduced, and the slope is used by dividing it into slightly sloping low steps (Morgan, 1986).

With terracing on arid and semi-arid sloping lands, products with high economic value can be grown easily, especially in the southeast Anatolia region. One of these products is vineyards. In addition to their economic value, grapes, which provide a wide range of benefits to human health, contain various antioxidant substances. Phytochemicals have attracted attention in recent years with their positive effects on health, especially their protectiveness against some types of cancer and heart diseases (Keskin et al., 2017). In addition, it is understood that melatonin has important functions in grapes. Considering that melatonin content is affected by many factors, especially genotype, it is important to examine the effect of melatonin in studies related to growth and development physiology, such as yield, quality, stress factors, resistance mechanisms in wide application areas of viticulture (Keskin et al., 2019).

Grape is one of the wealthiest fruit species for mineral and phytochemical content (Özrenk and Gökçen, 2020). Moreover, vines try to survive by developing many reversible or irreversible responses to UV light, which they perceive as a stress factor in their sessile nature. Thanks to these responses, vines can adapt to the factors of the environment they live in and survive (Gökçen and Keskin, 2020).

Olive is an immortal tree that has high economic importance, like grapes, and is capable of growing by terracing on sloping lands. Olive (*Olea europaea* L.) is a product of especially Mediterranean regions. Türkiye has great food consisting of olives, and olive oil Olives and food are used in Mediterranean dishes as an increase in food and flavor, but also because of their important roles for human health (Çetinkaya and Gökçen 2021).

Another definition, terraces are agricultural structures consisting of earthen ridges and canals built parallel to the contours and at certain intervals on sloping lands (Aydın, 1994; Çelebi, 1973; Çevik, 1998). Çanga (1995) defined terraces as earthen embankments established to cut the runoff water diagonally across the slope and to transfer these waters to a stable outlet at a non-erosive rate, as well as to reduce the length of the slope.

The benefits of terracing can be listed as follows:

- To make sloping, non-arable land suitable for agricultural activities,
- Creating new areas for crop production in areas where land is scarce,
- To prevent soil transport with erosion control in sloping lands,
- To make the land more easily workable and irrigable by reducing the slope degree.

Terracing is one of the oldest methods used to protect soil and water assets and to obtain agricultural land in areas where population density is high and agricultural area is low. Terracing works are carried out in areas where the land slope is high. In general, if the slope of the land is above 5%, it is accepted that the risk of erosion begins. In the case of 5-12% slope, it is possible to protect from erosion by plowing perpendicular to the slope, without the need for land terracing. The required slope range for terracing can be realized in a wide range between 15% and 60% (Dorren and Rey, 2018; Dunning, 1995).

Local conditions determine the size, shape and effectiveness of terraces. The productivity of a terrace system is enhanced by the application of additional conservation practices such as proper land preparation (contour plowing and planting), proper cultivation of crops (strip planting, etc.), and maintaining a permanent ground cover. The features that must be present in the land for terracing are as follows:

- Soil character is suitable for terracing,
- Decreased soil fertility,
- Land sloping is too much,

Depending on the plant growing conditions of the region, terraces take different names according to their purpose and construction. Terraces created to reduce the damage of surface water flow caused by precipitation in rainy regions are called "sloping terraces". In addition to the surface flow prevention and soil protection effects applied in regions with arid and hot growing conditions, the terrace shape that aims to retain water and leak the soil is called "flat terrace". Terraces, thanks to their unique structures, prevent the rapid flow of water on the surface and facilitate its infiltration into the soil, but if their maintenance is neglected, it can trigger soil degradation.

Terraces built on agricultural lands (Taysun, 1989; Çevik, 1998);

1- Ensure the removal of runoff waters in rainy areas without causing erosion,

2- To ensure that water is stored in the soil in arid regions,

3- They are built to make steeply sloping lands suitable for cultivation.

Construction purposes of terraces (Çevik, 1998);

1- Reduce soil erosion,

2- Ensuring sufficient water retention in the soil by leaking the surface flow into the soil,

3- By delaying the water collection time to prevent flood damage in the agricultural lands below or to bring sufficient water to the soil,

4- Prevent sediment accumulation,

5- Enable cultivated agriculture on steep sloping lands (example of Vietnam and Philippines),

6- It is to prevent the formation and progression of rifts.

Terraces are useful in arable soils. If the terrace project is well implemented and maintained, they reduce surface runoff and soil losses, which are the most important factors in protecting soil and water. In addition, terraces protect the topsoil and prevent the loss of plant nutrients. terraces; They are much more effective and beneficial if they are applied together with other methods such as crop rotation, stubble cover agriculture, contour farming and strip planting. For this reason, if soil improvement and productivity increase are desired, they should be applied together with other protective measures.

A land that is not suitable for growing field crops should be terraced. However, in areas where soil fertility has decreased too much, a terrace can be built to help establish a continuous meadow or forest cover (Çalık, 2021). The slope is the most important factor in terracing lands. The lands to be terraced should be suitable for the working styles of the existing tools and machines. The differences between the erosion susceptibility and permeability of various lands are important for terracing. Otherwise, the presence of negative factors in the land may expose the land to some hazards. The main factors limiting the use of terraces are land slope and soil depth. As the slope increases, soil loss due to erosion increases, and costs increase.

Erosion severity; It has a relationship with the degree of slope of the land, the length of the slope, the duration and intensity of precipitation, the characteristics of the soil, the type of plant and the agricultural methods applied. Soil depth and land slope are two important factors limiting the use of terraces. If the soil depth is insufficient, the required ridge height cannot be given for a suitable terrace. As the slope of the land increases, the soil losses due to erosion also increase. For these reasons, terraces should not be built in the following situations:

1- Very sandy, stony and very shallow lands,

2- Topographic conditions are unstable lands,

3- Other soil and water conservation methods such as crop rotation, stubble farming, contour farming, and strip cropping can prevent erosion lands.

Terracing is not recommended in soils with poor topography, very sandy, stony and shallow soils since soil and water conservation measures cannot control erosion well. Terrace determining, dimensions and spacing; according to precipitation intensity, soil sensitivity to erosion, and degree of the slope; it plays an important role in preventing the rainwater from turning into a surface flow and gaining the power to carry the soil, thus ensuring its infiltration under the ground.

Figure 2. Terraced field (Anonymous, 2022)

Table. 1 Slope, horizontal and vertical distances required on terraced lands (Hudson, 1981)

Average Slope (%)	Height difference between two terraces (m)	Horizontal distance between two terraces (m)	Average terrace length required for 1 ha land (m)
1	0.30	30.0	330
2	0.60	30.0	330
3	0.75	25.0	400
4	0.90	22.5	445
5	1.05	21.0	475
6	1.20	20.0	500
7	1.30	18.6	540
8	1.40	17.5	570
9	1.50	16.6	605
10	1.60	16.0	625
11	1.70	15.4	650
12	1.80	15.0	665

CLASSIFICATION OF TERRACES

The purpose of the terraces is to preserve the soil. Terraces do this by turning flood waters and transferring them harmlessly or absorbing water. Terraces are collected in 5 groups according to the way they are built:

2.1. Field Terraces

Field terraces are made in two ways.

2.1.1. Preventive and diverting terraces (flowing terraces)

Excess rainwater should be controlled in a way that does not cause erosion. Since the flood is desired to flow at a speed that will not cause erosion, attention should be paid to the channel that will safely drain the water rather than the ridge in these terraces (Figure 3).

Large, low-sloping but high-capacity terraces produce the desired results. The soil excavated on the surface of the field along the terrace channel is piled up at the desired cross-section to form a set starting from the lower edge of the terrace channel. Too high ridge is undesirable because the surface soil is more excavated, construction cost is high, and it makes soil tillage work more difficult.

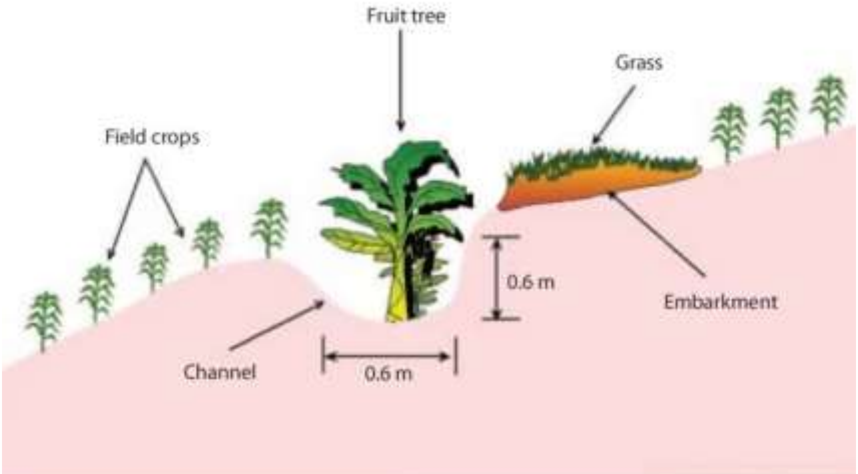

Figure 3. Cross-section of the terrace with flowing in places with high ground water (Mati, 2005)

2.1.2. Preventative and absorbent terraces (streamless terraces)

Erosion control and water saving are achieved at the same time thanks to these terraces. To increase the water absorption capacity of the terrace, it is necessary to collect the water coming from the surface flow in a large area as much as possible. For this, the slope of the land where the terrace will be built should be low, and the ridge should be made at a height where the water can spread over a large surface. The soil should be dug properly and some small pits should be created where water can accumulate. In these terraces, the ridge is more important than the channel. If maximum absorption is desired, the terrace should have a large channel capacity and the end ends should be closed in a horizontal position. Such terraces are suitable for regions with low rainfall and the soil's absorbing ability (Figure 4).

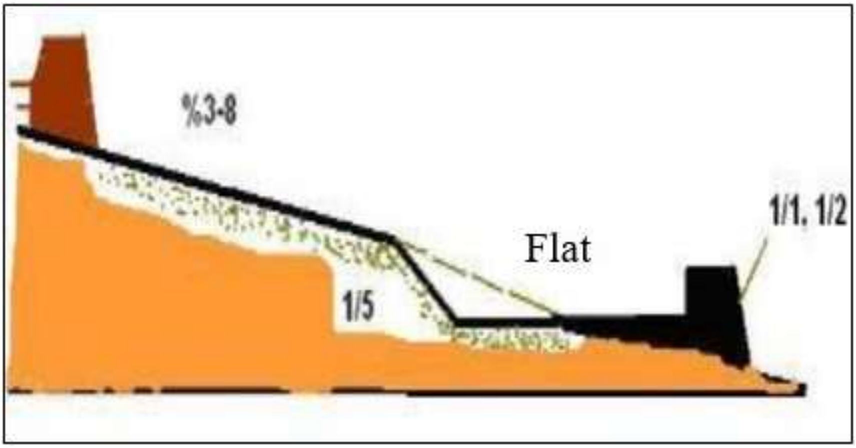

Figure 4. Streamless terraces (Anonymous, 2022b)

In rainy regions, to make the surface water flow caused by precipitation harmless the terrace shape created is called “sloping (deflective = flowing) terrace”. In arid regions and hot habitat conditions, the terrace shape, which aims to retain water and leak the soil, as well as prevent runoff and soil protection, is called "sloping (retaining = absorbent) terrace".

1.2. Step terraces

This type of terrace is established in places with a dense population and has to do agriculture on sloping lands to feed this population. Erosion losses are prevented by using step terraces, and it is possible to safely cultivate on very sloping lands (Calik, 2021). In areas where arable flat land is scarce, step terraces should be used as long as the sloping lands are allocated to plants that generate more income. Step terrace can become suitable for cultivation after 2-3 years of intensive processing and fertilization with farm manure and other green manures (Figure 5).

These terraces also called Algerian type in Southeast Anatolia “Step Terraces” are built with machinery. The terrace width is approximately 4 m. under the terraces used to stabilize parts according to the material; there are two types “earth terraces” and “stone terraces”. On pitches, up to 70% slope with terrace tillage can be done.

Figure 5. Step terrace (Anonymous, 2022c)

2.3. Interval terraces

These terraces, which are built at intervals of 20-40 meters on sloping lands, are called intermittent terraces or grodony-type terraces. These terraces are capable of handling the surface flow that will occur between the two terraces. The difference between these terraces from field type terraces is that soil cultivation is minimized by growing plants that need continuous and as little soil cultivation on the terraces as possible (Figure 6).

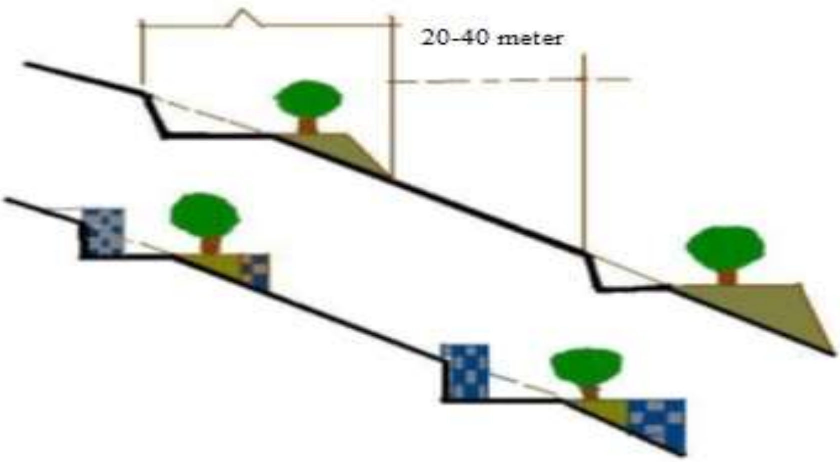

Figure 6. Grodoni (interval) type terrace (Anonymous, 2022a)

2.4. Trench terraces

These terraces are established to provide water conservation and erosion control and afforestation in the upper basin improvement works (Figure 7). While the trenches dug parallel to the leveling curves prevent erosion by holding the surface flow, water is also conserved for the seedlings to be grown. These are divided into three as flat-bottomed, backward-sloping-bottomed and deep trenches. In arid regions, ditches are not inclined.

2.5. Pocket terraces

Especially growing fruit trees such as olives on sloping lands, pocket-shaped pits are dug and a wall is built below these pits to provide water conservation and erosion control (Figure 8 and 9). The top view of the walls is crescent shaped.

The terrace shape used in areas with very high slopes (over 60%) is a bush reinforced or bushy terrace. bushy (reinforced) terraces; It is used to stabilize slopes that are sloping, sensitive to wind erosion, fine sandy structure, and thin material carried slopes. For this, a ditch is dug by giving a reverse slope to the excavation floor on the slope land and all kinds of branches are laid in bundles into this ditch.

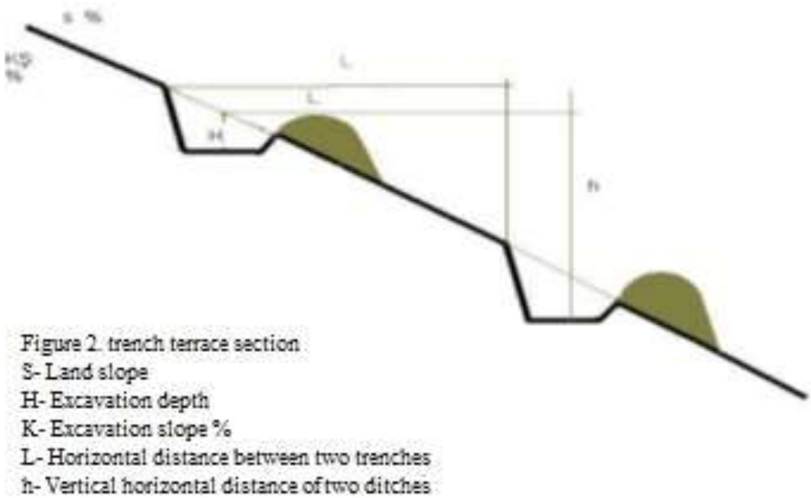

Figure 7. Trench type terrace (Anonymous, 2022)

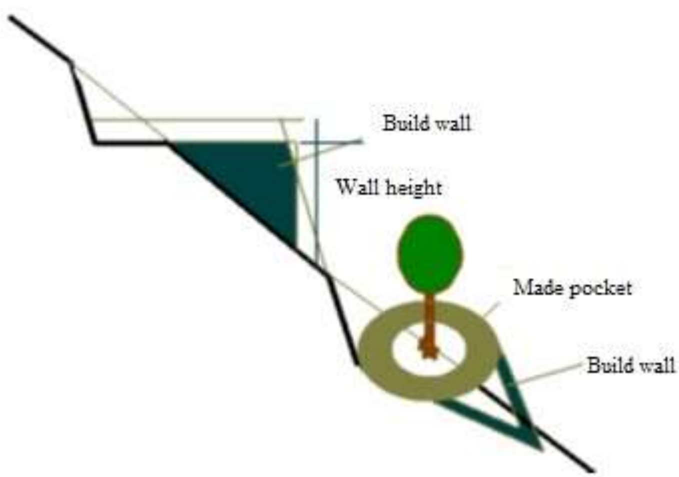

Figure 8. Pocket (dish) terrace - shrub bunched (reinforced) terraces (Anonymous, 2022b,d)

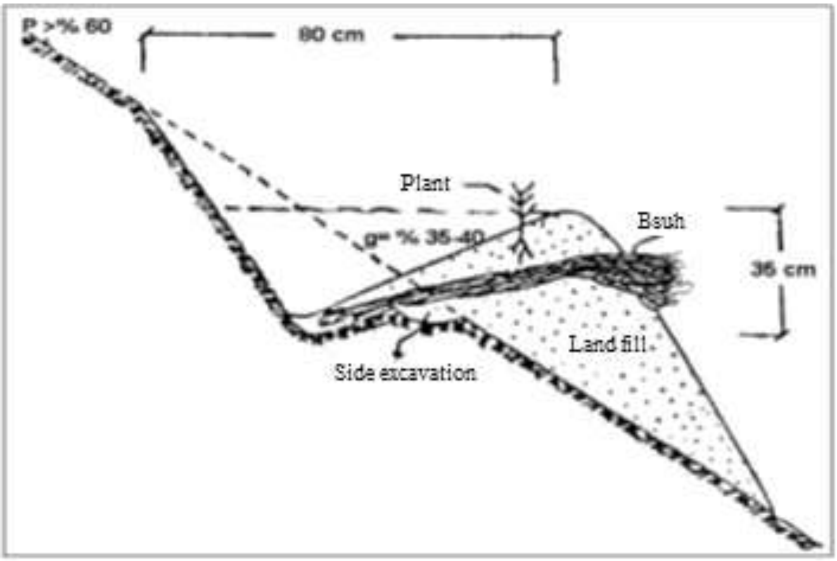

Figure 9. Shrub bunched (reinforced) terraces (Anonymous, 2022)

2. CONCLUSION

As a result, selecting, planning, and designing terrace types is very important on sloping lands, making it difficult to carry out some agricultural works such as tillage, irrigation, production of cultural plants, and machine harvesting. For these reasons, great care should be taken in making terracing projects and applying them to the land. The slightest mistake can cause enormous damage to terraced lands and other agricultural lands. We must be sensitive to the danger of erosion threatening large areas worldwide. When suitable conditions are provided on sloping lands, we should do terracing. We must cultivate these areas. In today's world, where food shortages and prices are increasing, it would be beneficial to evaluate our empty lands. We should grow fruit trees by terracing areas where agriculture cannot be cultivated. Terracing is a useful practice to protect soil and rehabilitate degraded soil.

REFERENCES

Anonymous (2022). http://www.ktu.edu.tr/dosyalar/silvikultur_9d4b3.pdf (Date of access: 12.06.2022).

Anonymous (2022a). https://tr.depositphotos.com/stock-photos/terracing.html A review of the effect of terracing on erosion. (Date of access: 18.06.2022).

Anonymous (2022b). https://topraktema.org | tema.org.tr (Date of access: 07.04.2022).

Anonymous (2022c). https://www.obmhaber.com/incirliovada-tarimsal-seki-teras-uygulamasi (Date of access: 25.05.2022).

Anonymous (2022d). https://tr.depositphotos.com/stock-photos/terracing.html (Date of access: 25.05.2022).

Çalık, A. (2020). The importance of organic agriculture and Şanlıurfa farmers' perspectives on organic products. *Turkish Journal of Agriculture-Food Science and Technology* 8(3): 728-732.

Çalık, A. (2021). Effects of different doses of plant mega minerals applied to different forage crops mixtures on dry grass crops and some quality criteria. *Bulgarian Journal of Agricultural Science* 27(4): 751-757.

Çalık, A. (2021). In semi-arid regions produced vetch of your plants water stress reactions. *International Journal of Environmental Trends (IJENT)* 5(2): 115-127.

Çanga, M.R. (1995). Toprak ve Su Koruma. A.Ü. Ziraat Fakültesi Yayın No: 1386. Ders Kitabı: 400.Ankara.

Çelebi, H. (1973). Teraslama. Atatürk Üniversitesi Ziraat Fakültesi Yayınları No: 91, Erzurum.

Çetinkaya, H. Gökçen, İ.S. (2021). Harvesting time substantially affects the squalene content in olive oils of Gemlik and Kilis Yaglik varietes. *Current Perspectives on Medicinal and Aromatic Plants* 4(2): 121-127.

Çevik, B. (1998). Toprak ve Su Koruma Mühendisliği. Çukurova Üniversitesi Ziraat Fakültesi Ders Kitabı, No: C-28, Adana.

Dorren, L., Rey, F. (2018). A review of the effect of terracing on erosion. *Soil Conservation and Protection for Europe* 97-108.

Dunning, T. (1995). Ancient maya terracing and modern conservation in the peten rain forest of Guatemala. *Journal of Soil and Water Conservation* 50(2): 138-145.

Gökçen, İ.S., Keskin, N. (2020). Asmanin UV (Ultraviyole) Stresine Yanıtlari. Ziraat ve Doğa Bilimleri Teori, Güncel Arastırmalar ve Yeni Eğilimler, Ivpe, Eds. Türkoglu, Nalan, Cantürk, Sevil.

Hudson, N.W. (1981). Non Technical Constraints on Soil Conservation in *Southeast Asian Regional Symposium on Problems of Soil Erosion and Sedimentation*, Asian Institute of Technology Eds. T.Tingsanchali and H. Eggers*).* 15-26.

Keskin, N., Gökçen, İ.S., Kunter, B. (2019). Asmanın Yaşam Döngüsünde Melatonin. Agricultural Sciences, 47. Current Research and Assesments for Agricultural Sciences, IVPE, Eds. Kunter Birhan, Keskin Nurhan.

Keskin, N., Gökçen, İ.S., Kunter, B., Cantürk, S. Karadoğan, B. (2017). Üzüm fitokimyasallari ve Türkiye'de yetiştirilen üzüm çeşitleri üzerindeki araştirmalar. *Turkish Journal of Forest Science* 1 (1): 93-111.

Mati, B.M. (2005). Overview of water and soil nutrient management under smallholder rain-fed agriculture in East Africa. Working Paper 105. Colombo, Sri Lanka: International Water Management Institute (IWMI).

Morgan, R.P.C. (1986). *Soil Erosion and Conservation* (Essex: Longman Group UK Ltd. 295.

Özrenk, K., Gökçen, İ.S. (2020). Differences To Chemical Constituent of Autochthonous Grape Cultivars in Siirt Province of Southern East Turkey. Agricultural and Natural Sciences: Theory, Current Research and New Trends, Eds. Kunter Birhan, Keskin Nurhan. Cetinje-Montenegro.

Taysun, A. (1989). Toprak ve Su Korunumu. Ege Üniversitesi Ziraat Fakültesi Teksir No: 92-3, İzmir.

CHAPTER 9

THE IMPORTANCE OF DRIP IRRIGATION TO REDUCE WATER CONSUMPTION IN ORNAMENTAL PLANTS AREAS

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1. INTRODUCTION

Water is a vital, renewable, but very limited resource for humanity. There are 1 400 000 000 km^3 of water on the earth's surface, including salt and fresh water. Only 350 000 000 km^3 of this amount consists of fresh water resources and corresponds to 2.5% of the total water amount. About 70% of the limited freshwater resources are located in glaciers and permanent snow in mountainous regions, and 30% is in the form of groundwater. Only about 0.3% is in rivers and lakes on earth. The total annual amount of usable fresh water for humans and ecosystems is 200.000 km^3, corresponding to less than 1% of the complete freshwater resources (UNEP, 2008). Our country also has a water potential of 112 km^3 as an annual renewable surface and underground fresh water source (DSİ, 2013).

Studies show that the need for limited fresh water resources is increasing day by day. According to FAO (2010) and UNWATER (2008) data, the rate of fresh water use in the current century has increased more than twice the population growth rate. Approximately 6.3 billion world population uses 54% of the renewable surface and underground water resources, and this value is predicted to increase to 90% in 2025 (UN/WWP 2003). In other words, after 2025, there will be almost no water left for ecological functions. He stated that while 1.1 billion people worldwide could not find enough water, 2.5 billion people could not reach healthy water. In addition to the figures, in the next 50 years, 1 billion more people in developing countries will be without water. Due to inadequate sanitation and hygiene, 160 million (40%) of 400 million school-age children endure abdominal pain and intestinal infections, resulting in over two million deaths yearly. When food production is limited due to insufficient water and deaths due to hunger are added, the number of people who die annually exceeds 40 million (Prinz, 2004). In the twentieth century, the world population has increased three times, and water use has increased six times. This change has taken a heavy toll on the environment. Water pollution has reached unprecedented levels, and more people cannot find sufficient water (Konukcu et al., 2007).

On the other hand, our country is in the position of a country experiencing water scarcity in the classification made according to the annual amount of water per capita, taking into account the current water potential situation. The yearly amount of usable water per person is around 1.519 m^3. The Turkish Statistical Institute (TUIK) has predicted that our population will be 100 million in 2030. In this case, it is estimated that the amount of usable water per capita for 2030 will be around 1.120 m^3 $year^{-1}$. It is possible to estimate the pressures on water resources with the effect of factors such as current growth rate and changes in water consumption habits. In addition, all these estimations will be possible if the existing resources are transferred 20 years later without destruction (DSİ, 2013). Otherwise, after 20-30 years, according to the classification of usable water amount per capita, our country will turn from a water-scarce country to a water-poverty country.

When the use of existing fresh water resources on a sectoral basis is examined, it is seen that a high rate of water is used in agricultural irrigation, such as 70%. It is used in industry with 22% and for domestic needs with 8% (UN/WWAP 2009). In addition to the effects of population growth and different sectors using water on the existing water potential, global climate change also poses an important threat. Even a 10% decrease in precipitation, which will occur with an annual average temperature increase of 1-2°C, may cause an average of 40-70% decrease in water resources per year (Postel, 1999). Climatic scenarios reveal that drought and thirst will increase worldwide due to global warming, negatively affecting our country and posing a great threat to our future. According to the estimates, as of 2050, the average temperature in our region in winter is 2°C; it shows that it will increase by 2-3°C in the summer months, and accordingly, the precipitation will decrease by around 15-25% (Kadıoğlu and Şaylan, 2004). Climate change and global warming have a great impact on agricultural production. The lack of precipitation experienced over the years causes a decrease in yield. Water needs to be provided for the plant nutrients to be effective. In some regions of Türkiye, agricultural production is continued without irrigation and fertilization. Water needed in agricultural production is provided by rainwater in semi-arid climate conditions (Bellitürk et al., 2019).

As can be understood from the forecasts made for the future, it is understood that the difficulties in the supply and use of water will increase exponentially according to the current conditions. Therefore, water-saving measures should be implemented. It is possible to meet the water used in domestic needs with a 10-15% savings in water use by the agricultural sector, which uses the most water on a sector basis. However, these savings can be possible with good programming and the application of agricultural irrigation. Some agricultural practices that can store water in the soil should be carried out in agricultural lands with low rainfall. Reduced tillage is an effective process for conserving soil water (Kuzucu and Dökmen, 2015). Soil surface mulching and rainwater harvesting also contribute to water saving and productivity in dry agricultural lands (Yazar et al., 2014; Kuzucu, 2021). The methods used to determine the irrigation program to be applied in agricultural irrigation are very important to achieve this goal. For this reason, the selected methods; should be environmentally friendly, easy to use, and accurately reflect the stress conditions of plants.

2. EFFECTIVE USE OF WATER IN RECREATION AREAS

Today, the amount of green space per capita in our big cities is generally 1m² or less. It is seen that this amount is decreasing gradually with the rapid population growth. However, our settlement areas with a population of over a million must have a minimum of 20m² green space standards per person. Therefore, protecting the increasingly narrow spaces between our settlements is necessary and creating new environments. One of the most important factors for protection landscape areas is to meet the water requirements of plants (Sarıkoç, 2007).

Landscape irrigation for private and public areas is estimated as one of the largest sources of urban water conservation potential. During the summer months, it is estimated that 50-70% of all municipal water is consumed in residential and public landscape areas. The traditional landscape characterizes large grass areas shaped by ornamental trees and shrubs. Especially in cities where irrigation is carried out with the water taken from the city network in parks and gardens, water consumption increases significantly in the summer months. This increase can sometimes be almost twice or even more than the winter months. This requirement of plants for water in summer depends on the fact that nearly all of the park and garden designs are created using exotic plants. This plant selection, which is made without adequate consideration of the existing environmental conditions, leads to water use to a large extent (Schneider, 2008).

The high water consumption in outdoor uses such as parks and gardens necessitated the development of new landscaping forms where water is used as little as possible in landscaping (Barış, 2007).

Various studies have been carried out on irrigation systems in landscape applications. Şahinler (1997) examined the types of automatic irrigation applied in landscape areas and the application in Bursa Metropolitan Municipality Soğanlı City Park. Erakın (2000) mentioned the irrigation systems used in landscape areas and their differences from each other. Onur (2002) discussed the design and application principles of the automatic irrigation system to be applied in the coastal arrangement of the city of Kocaeli. Seçkin and Çelik (2003) examined irrigation techniques, the importance and water consumption of grass plants in park areas, irrigation water requirement, irrigation interval, and duration. Yıldırım (2003) mentioned the types of automatic irrigation applied in recreation areas and their applicability. Aslıoğlu (2005) examined the importance and irrigation types applied in recreational and sportive green regions. Demirel (2005) examined the types of different sprinkler irrigation heads used in landscape projects and determined their differences and performances from each other. Sarıkoç (2007) revealed the water consumption, irrigation water requirement, irrigation interval, and irrigation duration of the grass plant in the park areas of our country in three different climatic regions (Antalya, Ankara, and Trabzon).

Achieving the expected benefit from irrigation depends, first of all, on the use of appropriate irrigation methods and techniques. In this context, automatic irrigation systems have been preferred in landscaping applications in recent years. Some basic principles should be considered when using these methods. Among the most important of these; are the less water needed, the irrigation materials not occupying much space, and the visuality. In any case, new irrigation techniques, together with visuality in limited irrigation programs for today's conditions, effectively reduce irrigation costs.

Plants, used as living materials, need water to continue their lives, as in all living things. The water required is mainly taken from the soil by plant roots. Some of the water taken up by plants is used to make various compounds and primarily for photosynthesis. A very important part of it is given to the atmosphere through perspiration. The amount of water remaining in the plant and used in various physiological processes is too small to be considered in addition to the amount of water given to the soil through transpiration (Sarıkoç, 2007).

For this purpose, the amount of water to be delivered to plants and plant water consumption gain importance. There are many factors that affect plant water consumption (Table 1). As temperature, wind speed, and sun exposure increase, plant water consumption increases and decreases as relative humidity increases.

Irrigation methods are generally considered surface and pressurized. Pressure irrigation (sprinkling and drip) is generally used in recreation areas and is controlled by automation (Orta, 2009). Here, we will talk about the drip irrigation method used in areas that cannot be irrigated with the sprinkler irrigation method and in limited water conditions.

Table 1. Factors affecting plant water consumption

1. Climate factors	2. Soil factors	3. Plant factors
Solar radiation	Soil moisture	Plant species
Temperature	Treatment status of the soil	Development circuit
Relative humidity	Flora	Growing season
Wind		
Daytime hours		

3. USE OF DRIP IRRIGATION SYSTEM IN RECREATION AREAS

The basic principle in the drip irrigation method is to transmit the required small amount of irrigation water to the root zone at frequent intervals without creating tension due to moisture deficiency in the vine. This irrigation system successfully irrigates trees, shrubs, ground covers, flowers, vineyards, greenhouse plants, and vegetables. For the success of the method, it is important to analyze the area to be irrigated in the best way, planting design, design of the irrigation system, the installation of the irrigation system, the determination of the irrigation periods, and the maintenance of the system (Öztürk, 2008).

Water is the most consumed natural resource in the maintenance works of green areas. With faulty irrigation methods, huge amounts of water are wasted without being used by plants. With the use of high-pressure running water, some water flows off the surface of the soil, and some of it evaporates from the soils that are not covered with leaves and plant residues and are open to the sun and wind. The greatest amount of water loss comes from too much water too often. Excessive irrigation damages plant roots and adversely affects plant growth. At the same time, it causes the nutrients in the soil to be washed away from the plant root area and go deep into the soil. This situation increases the possibility of contamination of groundwater. Similarly, water runoff caused by over-irrigation can carry polluting fertilizers and pesticides into lakes and streams. Pollution or waste of high-quality water by faulty irrigation methods should be eliminated by using effective irrigation techniques. The irrigation system should be well-planned, and irrigation should be managed well. Not all plants need the same amount of water. Lawn areas should be watered separately from other areas. Plants with similar water requirements should be collected in the same regions. Irrigation should be done according to the conditions of the plants rather than sticking to a certain schedule. Except for lawn plants, most plants do not need regular watering for several years after planting. Lack of regular irrigation encourages deep-root development in trees and shrubs and makes plants resistant to drought (Yazgan and Özyavuz, 2008). Generally, drip irrigation system uses approximately 20% less water than sprinkler irrigation systems (Addink, 2005).

Some advantages of this method are:

1. The need for irrigation water is reduced, as only the area where the roots develop is wetted instead of the entire land.
2. Since the wet surface is under the plant crown, plant water consumption is reduced due to evaporation.
3. Plant nutrients and pesticides can be applied effectively in the system.
4. In saline soil and/or brackish water conditions, reliable irrigation can be done as the salt will be carried out of the wet wall.
5. Since the entire area is not wetted, weed control is facilitated, and since the above-ground part of the plant is not wetted, the control of plant diseases and pests becomes easier.
6. It can be applied in sloping and shallow soil conditions without causing erosion and drainage problems.
7. Operating costs are lower than the sprinkler irrigation method due to low operating pressure.
8. It provides controlled and safe irrigation in recreational areas, narrow bands that are not uniformly shaped.
9. It is successfully used to apply additional irrigation water to deep-rooted shrubs, shrubs, and tree-like plants in grass-intensive recreation

areas.

10. It is an ideal irrigation method for ornamental plants grown in pots.

In addition to these advantages of drip irrigation, it has disadvantages such as clogging of drippers and the salinity it will create in the soil under saltwater conditions. Drip irrigation is not preferred in lawn areas because the laterals are on the surface and pose a problem for the shape. In addition, since all the grass areas will have to be wetted, the laterals will be laid so frequently, especially in light-textured soil conditions, that the development and appearance of the grass will be difficult (Mid, 2009).

4. ELEMENTS OF DRIP IRRIGATION SYSTEM

Control unit: In this unit, where the pump, pressure regulator, fertilizer tank, and filter are located, It is ensured that the amount of water to be given and the pressure are adjusted, the water is filtered, and the desired nutrients are mixed with the water. It consists of the control unit, pump, filter, pressure and water application time regulating devices, a water clock, and fertilizer tank, which are created close to the water source (Figure 1) (Bağdatlı, 2006; Hakgören, 1996).

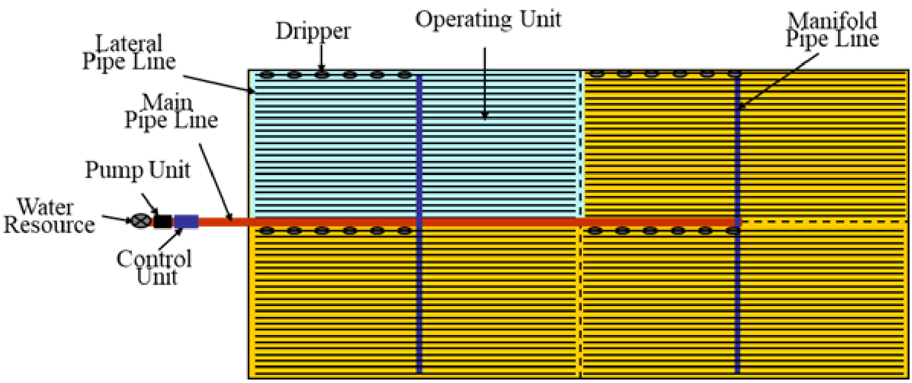

Figure 1. Elements of the drip irrigation system

Main pipeline: It is the elements that transmit the water coming out of the control unit to the side pipes. It should be of a suitable diameter for the required irrigation water flow and a sufficient length for the water transmission distance. They are usually made of rigid PVC and are buried underground.

Side pipeline: They take the water from the main pipeline and transmit it to the laterals. These, too, are usually embedded and made of either rigid PVC or soft PE.

Laterals are pipes with drippers inside or on them and are generally made of soft plastic. 16 and 20 mm diameter ones are more common in the market.

Drippers: Drippers are water sources made of plastic, spaced along the laterals and applying a low flow rate with low pressure. The equipment provides the application of the system and gives the water to the soil with a constant flow under atmospheric pressure. Figure 2 shows the positioning of the drippers around the tree and the wetting areas. Wet area forms along the lateral of the plants to be irrigated are given in Figure 3.

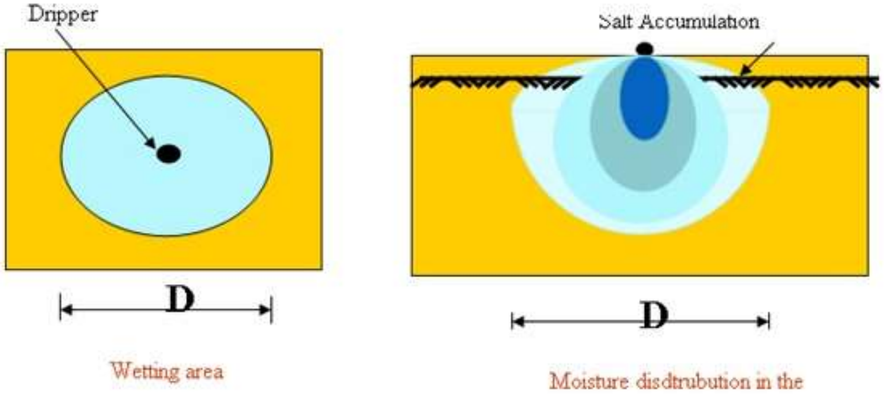

Figure 2. Wetting area and moisture distribution pattern of a dripper

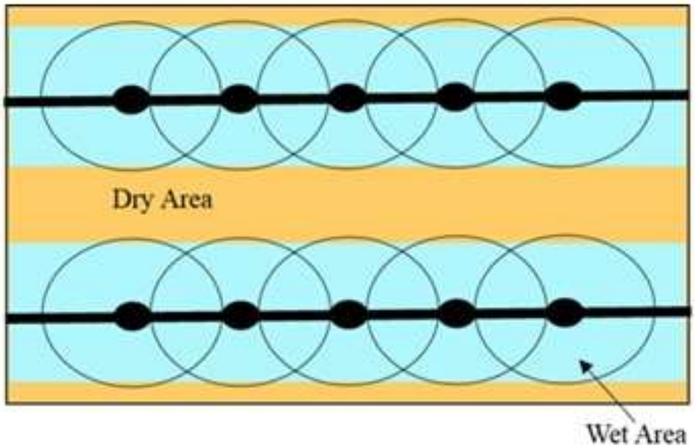

Figure 3: Wet area along lateral

5. CONCLUSION AND RECOMMENDATIONS

Drought, water scarcity, and scorching heat have been effective in Türkiye, especially for the last ten years, showing that global warming will be a part of life. The effects of climate change and global warming will emerge in various ways and force all living things to adapt more (Atik and Sayan, 2007).

If there is no consensus on fundamental measures to stop global warming at the international level, in the following years; drought, scorching heat, and the salinity of soil and irrigation water will increase, and these three factors will play a decisive role in the cultivation of plants (Aydeniz, 2008).

Drought and extreme heat will further hinder the efficient cultivation of plants. Therefore, to solve the problem, by carrying out multidimensional and careful adaptation studies, a new plant production pattern suitable for new conditions should be prepared, and information should be given about which species would be more suitable to be grown (Aydeniz, 2008). In dry production conditions, soil moisture is very important, from seed germination to product yield. In order to get high-quality and abundant products, it is necessary to soil moisture conservation in non-irrigation agriculture areas ((Kuzucu, 2017; Kuzucu, 2019).

In the design phase, where aesthetic concern and functionality come to the fore in landscape architecture, it is important to adopt approaches defined as “naturalistic” and based on the re-creation of nature. One of the issues that landscape designers are interested in is the reorganization of natural processes. It is necessary to preserve the areas outside the buildings in their natural state as much as possible, to choose drought-resistant ornamental plants and/or to natural plant species specific to the region when choosing plant material, to use species with similar irrigation needs together, and to rank according to the water needs of the plants. Wide grass surfaces should be avoided unless necessary, drought-resistant grass species should be used for football fields and golf fields, which have a large place in water consumption, and perennial ground cover shrubs should be used instead of seasonal flowers (Karagüzel, 2007).

Suppose a general evaluation is made on the landscape arrangements made in the Central Anatolian region. In that case, it can be observed that the arid climatic conditions and water needs are not considered in the plant species used. It is understood that too much grass is used in the applications, and soil conditions are not considered. As a result of the intensive use of grass, the need for water increases, and if sufficient irrigation is not done, yellowing and burning can be observed in the grass areas. In this context, the first thing to do is to develop different designs for each area. “Naturalistic” arrangements, an approach accepted worldwide, can be taken as a basis for xeric landscape arrangements to be realized in Central Anatolian conditions. Natural plant species found in the areas should be protected and used. In the case of Central

Anatolia, as in many other regions, the uncontrolled dense structuring within the settlements and some negative consequences perceived by the activeness in their surroundings cause the problem of the decrease in urban and regional scale open green areas, which will ensure the healthy conduct of human and environmental relations. In addition, the increasing population with this dense construction is rapidly destroying water resources. Global warming and climate change negatively affect agriculture in dry agricultural lands. In dry regions with low rainfall, water should be used sparingly, and alternative practices that can save water should be started (Kuzucu et al., 2016). Plant species that have the same or close water requirement should be used. Central Anatolia region has plant species resistant to extreme summer temperatures with its arid/semi-arid climate. Particular attention should be paid to using natural plant species in the region, and traditional design approaches based on foreign plants should be abandoned. In Central Anatolian climate conditions, steppe plants, bulbous plants, and perennial flowering plants can be used from these plant species. Grass use should be minimized. Instead of grass types, ground cover and low shrubs should be preferred. The most suitable garden irrigation systems should be placed in irrigation, and the appropriate irrigation pattern, time, and method should be determined.

REFERENCES

Addink, S. (2005). Cash for grass – A cost effective method in conserving lanscape water? *University of California, Riverside, Turfgrass Research Facility, http://ucrturf.ucr.edu/.*

Asıllıoğlu, F. (2005). *The importance of ırrigation and ırrigation systems in recreational and sportive green areas in terms of landscape architecture*. (Master's thesis) Ankara University, Ankara.

Atik, M., Sayan, S. (2007). Antalya and drought. *Antalya Chamber of Industry and Commerce Publication Organ, 236:6-10, Antalya.*

Aydeniz, O. (2008). Growing new plant species according to changing conditions in our Country. *Cyprus Newspaper.*

Bağdatlı, C. (2006). *A research on drip ırrigation systems applied in vegetable gardens around Konya*. (Master's thesis) Selcuk University, Konya.

Barış, E. (2007). Drought landscape. *Journal of Science and Technology* 478, Tübitak, Ankara.

Bellitürk, K., Kuzucu, M., Baran, M.F., Çelik, A. (2019). Antep Fıstığında (*Pistacia vera* L.) kuru koşullarda gübrelemenin verim ve kaliteye etkileri. *Tekirdağ Ziraat Fakültesi Dergisi* 16(2): 251-259.

Demirel, K. (2005). *A study on determining the performance of different sprinkler ırrigation heads used in landscape projects.* (Master's thesis) Çanakkale Onsekiz Mart University, Çanakkale.

DSİ (2013). http://www.dsi.gov.tr /toprak-ve-su- kaynaklari (Date of access: 28.01.2013)

Erakın, A. 2000. *Irrigation systems used in landscape planning studies.* (Master's thesis) Ege University, İzmir.

FAO (2010). http://www.faostat.com/2010 statistics (Date of access: 29.01.2013).

Hakgören, F. (1996). Irrigation (planning and projecting principles). *Akdeniz Univ. Arrow*. 67:229-239, Antalya.

Kadıoğlu, M., Saylan, L. (2004). Global climate changes and our water resources. Chamber of architectures, Istanbul Metropolitan Branch. *Istanbul and Water Symposium, 8-9 January, Istanbul.*

Karagüzel, O. (2007). Grass and groundcover plants textbook. *Akdeniz University, Faculty of Agriculture, Department of Landscape Architecture, Antalya.*

Konukcu, F., İstanbulluoğlu, A., Kocaman, İ., Albut, S., Gezer, E. (2007). Global water crisis: present, future and preventable possibilities, *22 March World Water Day Events, SUSED* 1: 24-49, Tekirdag.

Kuzucu, M., Dökmen, F. (2015). The effects of tillage on soil water content in dry areas. *Agriculture and Agricultural Science Procedia* 4: 126-132.

Kuzucu, M., Dökmen, F., Güneş, A. (2016). Effects of climate change on agriculture production under rain-fed condition. *International Journal of Electronics Mechanical and Mechatronics Engineering* 6(1): 1057-1065.

Kuzucu, M. (2017). The economic evaluation of water harvesting techniques in dry agricultural areas. *Journal of Multidisciplinary Engineering Science and Technology* 4(9): 8267-8270.

Kuzucu, M. (2019). Effects of water harvesting and organic fertilizer on vineyard (*Vitis vinifera* L.) yield and soil moisture content under arid conditions. *Bangladesh Journal of Botany* 48(4): 1115-1124.

Kuzucu, M. (2021). Importance of mulching ın dry agricultural areas for soil moisture storage. *International Journal of Environmental Trends (IJENT)* 5(1): 16-27.

Onur, B.E. (2002). *Irrigation system design of Kocaeli province coastal arrangement. (*Master’s thesis) Istanbul University, Istanbul.

Orta, H. (2009). Irrigation in recreation areas, *Namık Kemal University, Faculty of Agriculture, Department of Agricultural Structures and Irrigation, Tekirdag.*

Öztürk, T. (2008). Economical use of water in landscapes: drip irrigation systems. *Irrigation and Salinization Conference, Şanlıurfa.*

Postel, S. (1999). Last oasis. Facing water scarcity. *Worldwatch Institute. Washington.*

Prinz, D. (2004). Water and development - the challenge ahead, *Ewra Symposium on Water Resources Management: Risk and Challenges for the 21. Century.* 2-4 September Izmir.

Sahinler, Ç. (1997). *Landscape irrigation design and Bursa Metropolitan Municipality Soğanlı City Park application.* (Master's thesis) Uludag University, Bursa.

Sarıkoç, E. (2007). *Irrigation methods used in landscape areas and application of plant water consumption models in three different climate regions of Turkey.* (Master's thesis). Karadeniz Technical University, Trabzon.

Schneider, J. (2008). *A look into water conservation: an evaluation of landscape water regulations* (Master's thesis). Kansas State University, Manhattan, Kansas.

Seçkin, Ö., Çelik, H. (2003) Introduction to irrigation. *Istanbul University Publication No: 4421, Forestry Faculty Publication No: 472, Istanbul.*

Un/Wwap (2009). Climate chance and water. An Overview from The World Water Development Report 3: Water in A Chancing World. www.unesco.org/water/wwap (Date of access: 29.01.2013).

Un/Wwp (2003). World water development report, water for people, water for life. Unesco, Berghahn Book.

Unep (2008). United Nations Environment Programme. Annual Report. http://www.unep.org/pdf/annualreport/2008/annualreport2008_en_web.pdf (Date of access: 31.01.2013)

Unwater (2008). United Nations Water. http://www.unwater.org/ Documents.Html (Date of access: 28.01.2013).

Yazar, A., Kuzucu, M., Celik, I., Sezen, S.M., Jacobsen, S.E. (2014). Water harvesting for improved water productivity in dry environments of the Mediterranean region case study: pistachio in Turkey. *Journal of agronomy and crop science* 200 (5): 361-370.

Yazgan, M.E., Özyavuz, M. (2008). Xeriscape, a new system in landscape architecture. Unpublished Lecture Notes, Ankara.

Yıldırım, M. (2003). Rules to be followed in recreation area ırrigation systems. *2nd National Irrigation Congress, Proceedings, 16–19 October 2003*. Kuşadası-Aydın, 134–142.

CHAPTER 10

THE ROLE OF BIOSTIMULANTS IN VITICULTURE

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1. INTRODUCTION

Today, one of the most important questions that the world needs to answer is how to feed the world population, which is likely to exceed 9.3 billion by 2050, healthily, and adequately (Searchinger et al., 2013). Maintaining food production for a growing world population without compromising natural and environmental resources such as ecosystems, climate, and water for future generations is one of the greatest challenges for agricultural science (Searchinger et al., 2013; Colla et al., 2015).

Popescu (2019) has indicated that intensive agriculture reached a critical point, and the negative impacts of this activity are now resulting in irreversible global climate change and the loss of many ecosystem services. Some researchers (Tilman et al., 2011; Canellas et al., 2015) expressed that some abiotic stresses caused by low and high temperatures, salinity, drought, heavy metal toxicity, nutrient deficiency, and overuse of synthetic fertilizers could reduce plant production and lead to important uncertainty in food security. Therefore, new approaches to help promote sustainable agriculture are required. Achieving these goals may be possible by encouraging the use of natural products known as plant bio stimulators for scientists and producers (Calvo et al., 2014; Ruzzi and Aroca, 2015; Savvas and Ntatsi, 2015; Popescu, 2019). Biostimulants could reduce fertilizer use, increase drought and heat stress resistance, and improve yield, plant growth, and physiology (Paradikovic et al., 2011; Turan et al., 2021). Fertilizer use in modern agriculture is highly inefficient; much of the applied fertilizer is released into the environment, causing pollution. One way fertilizer use could be reduced without preventing plant nutrition is to enhance nutrient uptake via biostimulants (Halpern et al., 2015).

According to Du Jardin (2015), the word “biostimulant” was firstly used by Kauffman et al. (2007) in a peer-reviewed article. Biostimulants are materials other than fertilizers that promote plant growth when applied in low amounts. Many researchers have increasingly used the word biostimulant in the following years by expanding the scope of substances and modes of action (Du Jardin, 2012; Calvo et al., 2014; Külahtaş and Çokuysal, 2016). Despite recent efforts to clarify the regulatory status of biostimulants, there is no legal or regulatory definition of plant biostimulants anywhere in the world, including in the European Union and the United States (Du Jardin, 2015). Therefore, it is getting hard to list and classify the substances and microorganisms covered by the "biostimulant" concept in a detailed and precise manner.

Du Jardin (2012), considering more than 250 articles that use the word biostimulant in the title or abstract, suggested dividing biostimulants into eight groups (1) humic substances, (2) complex organic materials, (3) beneficial chemical elements, (4) inorganic salts including phosphite, (5) seaweed extracts, (6) chitin and chitosan derivates, (7) antitranspirants, and (8) free amino acids and N-containing substances. However, the former bibliographic analyses did not include the biostimulant effects of beneficial microorganisms (i.e., arbuscular mycorrhizal fungi, other beneficial fungi like *Trichoderma* spp., and plant growth-promoting rhizobacteria).

Colla and Rouphael (2015) stated that plant biostimulants are usually applied to high-value crops, mainly greenhouse crops, fruit trees, open-field vegetables, flowers, and ornamentals, to increase yield and quality sustainably. According to the authors, while biostimulant products were initially used in organic production, they are now attracting more and more attention in conventional agricultural production, considering economic and sustainability concerns. According to The European Biostimulants Industry Council (EBIC) (2022), most market analysts report that the European biostimulants market accounts for roughly half of the global market. Estimates of the European market value range around USD 1.5-2 billion in 2022. The compound annual growth rate reported is 10-12%.

2. BIOSTIMULANTS USED IN VITICULTURE

Grapes (*Vitis vinifera* L.) in the whole world have been used for: (i) wine production (57%), (ii) table grapes (36%), and (iii) raisins (7%). Due to an approximate value of over US$70 billion, grapes are the world's third most valuable horticultural crop after potatoes and tomatoes (Alston and Sambucci, 2019). According to the OIV statistics of 2018, more than 7.8 million metric tons of grapes have been produced on 7.45 million hectares. Of this total grape production, 57% was used for wine, 36% for table grapes, 7% for raisins, and less than 1% for grape juice, brandy, or transformed into vinegar. In the world, 37% of grapes are produced in Europe, 34% in Asia, and 19% in America. In terms of vineyard area, Spain, France, China, Italy, and Türkiye are in the top five, while China, Italy, USA, Spain, France, and Türkiye are in the top six in terms of gape production (OIV, 2019).

Viticulture, which has a significantly high commercial volume in terms of raisins, table, and wine grape production in the world and Türkiye, is nowadays faced with many problems, such as salinity, metal toxicity, chemical fertilizers, and synthetic pesticides. Against these, in viticulture, environmentally friendly practices such as integrated pest management and organic and biodynamic farming have been done to control synthetic fertilizers, pesticides, and plant growth regulators typically used in conventional farming (Samuels et al., 2022).

In addition to the abovementioned problems, high temperature, drought, solar radiation, and atmospheric CO_2 concentration are the abiotic factors that change due to global warming. These abiotic factors affect the synthesis and degradation of primary (sugars, amino acids, organic acids, etc.) and secondary (phenolic and volatile aroma compounds and their precursors) metabolites, also grapevine physiology, phenology, and grape composition, and finally, wine, wine microbiology, chemistry, and sensory aspects directly or indirectly (De Orduna, 2010; Rienth et al., 2021). According to Bernardo et al. (2018), biostimulant use in the viticulture of the world and Türkiye is increasing daily. Du Jardin (2015) also indicated that table and wine grapes are among the main crops on which biostimulants are applied in Europe.

Considering the use of biostimulants as a mitigation strategy for biotic and abiotic stresses in the vine, they are generally classified similarly to those in other plants. This review aimed to provide information about the general mechanisms of action and research results of biostimulants used in viticulture, considering Du Jardin (2012) and the main categories generally accepted by some other researchers (Du Jardin, 2015; Gutiérrez-Gamboa et al., 2019; Rouphael and Colla, 2020; Monteiro et al., 2022; Samuel et al., 2022).

Humic substances (humic and fulvic acids)

Today, one of the approaches that can be effective in ensuring the sustainability of agriculture against the negative effects of incorrect agricultural activities and global climate change applied as a result of intensive agriculture is the use of plant biostimulators derived from humic substances. Humic substances (HS) are sources of organic carbon produced by the biodegradation of organic matter formed by chemical and biological conversions of animals and plants (Canellas et al., 2015).

Humic acid (HA) has been shown to have a hormone-like activity that stimulates plant growth and yield by acting on mechanisms involved in cell respiration, photosynthesis, protein synthesis, water and nutrient uptake, and enzyme activities (Chen et al., 2004; Ferrara and Brunetti, 2010). Humic acids have indirect and direct effects on plant growth. Indirect effects are mainly exerted through properties such as enrichment in soil nutrients, increased microbial population, higher cation exchange capacity, and improved soil structure. In contrast, direct effects are various biochemical actions exerted at the cell wall, membrane, or cytoplasm, mainly hormonal (Chen et al., 2004).

According to Mostafa et al. (2017), fulvic acid (FA) is highly beneficial to both plants and soil because 1) It is important for increasing microbial activity. 2) it promotes nutrient uptake as a chelating agent and enhances vegetative development, nutritional status, and leaf pigments.

El-kenawy (2017) reported that fulvic acid application of 500 mg L^{-1} concentration affected the acidity and total phenols in Thompson Seedless. Foliar application of fulvic acid, Mg, and K provided the highest bud burst, vegetative growth, yield, and total anthocyanin content in berry skin compared to the control (Mostafa et al., 2017). Ferrara and Brunetti (2010) showed that foliar application of humic acid at 100 mg L^{-1} at full bloom significantly increased berry size and other quality parameters.

Kök and Bal (2016) investigated the effects of foliar applications at the concentrations of 1000 and 2000 ppm of seaweed (SW) and humic acid (HA) on cv. Riesling. The best quality was obtained from 1000 ppm concentration 30 days after veraison and 15 days after veraison and veraison, respectively. The results showed that SW and HA could modify the biochemical characteristics in cv. Riesling.

El-Boray (2021) and El-Boray et al. (2013) studied the effects of humic acid, bio-fertilizers, and microelements on the leaf mineral contents of cv. King's Ruby. Results showed that HA (3 g vine^{-1}), bio-fertilizers (7.14 mL vine^{-1}), and micro-elements significantly activated the macro element absorption. Besides, adding HA at 3 g vine^{-1} with bio-fertilizers and micro-elements also activated micro-elements uptake of grapevines. On the other hand, the control gave the lowest concentration of macro and micro-elements compared to the other treatments in both seasons.

Belal (2015) investigated the effects of foliar application of humic acid (HA), effective micro-organisms (EM), and three antioxidants (ascorbic acid, citric acid, and ascorbic+citric acid) on vegetative growth, leaf mineral content, yield, and fruit quality of King's Ruby grape cultivar. The results showed that HA application on soil provided the highest cluster weight, yield, berry characteristics, vitamin C, and leaf chlorophyll content, N, P, and K content in leaf petioles compared to EM application.

In the study of Abdel-Salam (2016a), humic acid, ascorbic acid, and citric acid were applied at 300, 2000, and 2000 ppm, respectively, on clusters and leaves of Ruby seedless grapevine. The results demonstrated that all the treatments improved all the parameters compared with the control. The combination of humic, ascorbic, and citric acid gave the best effect compared with the other treatments in the two successive seasons.

Amino-acid and other nitrogen-containing substances (protein hydrolysates)

Protein hydrolysates (PHs) are important plant biostimulants based on mixtures of peptides and amino acids mainly produced by enzymatic and/or chemical hydrolysis of proteins from animal or plant-derived raw materials

(Colla et al., 2015). Glycine betaine (GB) is an N-trimethyl glycine derivative compound that belongs to the quaternary amines. It is found in many bacteria, plant, and animal species (Monterio et al., 2022). GB since it is naturally synthesized, readily available, nontoxic, cheap, and with increasing evidence from in vivo and in vitro studies of the physiology, biochemistry, genetics, and molecular biology of plants, it is strongly suggested that GB has an important function in plants exposed to environmental stress. It plays an adaptive role in osmoregulation and protecting the sub-cellular structures in stressed plants (Mickelbart et al., 2006; Hussain Wani et al., 2013; Hayes et al., 2020). Production of osmolytes such as proline and glycine betaine (GB) has an important place in the reaction of plants to drought. Therefore, plant resistance to drought could be increased through the exogenous application of these materials. Zamani et al. (2013) experimented on four grape cultivars (Khushnav, Peykani, Perlette, and Flame Seedless) under drought stress, which was irrigated with 70% of the required water. In the study, proline (10 mM) and GB (15 mM) were sprayed on the grapevines at four phenological stages. Results indicated that proline and GB significantly affected leaf relative water content, canopy temperature, chlorophyll content, leaf area, and soluble carbohydrate in all the cultivars. Finally, the authors concluded that GB was more effective than proline in increasing resistance to drought stress in grapevine.

Mickelbart et al. (2006) found no genetic variation in GB levels among field-grown Chardonnay or Pinot noir clones and found the endogenous GB levels to be very low. The authors detected growth cessation and phytotoxicity at 100 or 200 mM concentrations of GB and suggested working at GB concentrations below 50 mM in future studies. Observing the effects of foliar GB applications on cv. Alphonse Lavallée grown under deficit irrigation, Jalil

(2017) stated that the GB application could be recommended as an environmentally friendly and sustainable practice under water deficit.

Bernardo et al. (2018) determined that GA application at a concentration of 7.65 g L^{-1} in cv. Pinot Noir increased leaf area and leaf-specific weight. In pre-harvest foliar spraying of table grapes (*V. vinifera* L. cvs. Olhoghi and Rishbaba) with putrescine and spermidine (0, 1, 2 mM), berry parameters were evaluated after 0, 25, and 55 days of storage at 1.5 ± 1 °C and 90 ± 5% R.H. (Mirdehghan and Rahimi, 2016). According to the authors, although softening, fungal infection, and weight loss increased during cold storage, the change rate was significantly delayed in putrescine and spermidine-treated clusters. Besides, applying putrescine and spermidine maintained phenolic content, antioxidant activity, and anthocyanins at the end of storage. In cvs. Garnacha, Graciano, and Tempranillo, urea application at a 1.61 g L^{-1} dose changed the amino acid content of grapes (Gutiérrez-Gamboa et al., 2018).

Bassiony et al. (2018) have aimed to determine the effects of foliar applications of putrescine at 0.0, 1.0, and 2.0 mM, salicylic acid (SA) at 0.0, 2.0, and 4.0 mM, and the combination of both on yield, berry quality and storability of cv. Flame Seedless. The results revealed that the combination of treatments containing putrescine at 2.0+SA at 4.0mM increased total yield and improved cluster quality.

A study was conducted to determine the effects of exogenous melatonin treatment on phenolic compounds and antioxidants of grape berries and wine in cv. Merlot (*Vitis vinifera* L.) (Meng et al., 2019). The results showed that melatonin treatment increased the concentration of cyanin-3-O-glucoside, peonidin derivatives, (+)-catechins, (−)-epicatechins, and flavonols in both cultivars and wines. The authors stated that the study could provide a practical strategy for changing the phenolic composition of grapes and wine.

According to Bernardo et al. (2018), despite the contrasting results about the effects of foliar nitrogen application on the amino acid content of must, most studies reported an increase in must amino acids and yeast available nitrogen content in grapevines.

In the study of Champa et al. (2015), spermine application in Flame seedless table grape cultivar (*Vitis vinifera* L.) indicated that at 1.0 mmol/L maintained approximately 20% higher firmness over control and weight loss reduced by ≈2 fold with 0.5 and 1.0 mmol/L doses of spermine. Besides these, 1.0 mmol/L spermine treatment increased berry color and anthocyanins and extended storage life by 15 days compared to the control.

Chitosan and other biopolymers

Chitosan is a natural, non-toxic long-chain polymer of N-acetyl-glucosamine obtained by deacetylation of chitin from shrimps and crustaceans shells, mollusks, fungi, and algae cell walls and insects (Orzali et al., 2017; De Bona et al., 2021; Monterio et al., 2022). Monterio et al. (2022) stated that chitosan has antimicrobial properties and can elicit plant defense against pathogens. It is a polymer that forms a semipermeable film around plant tissues, inhibiting several pathogens and inducing defense response mechanisms in the host tissues. Some studies have shown that it improves the grapevine defense responses, yield, and quality under stress conditions.

Pichyangkura and Chadchawan (2015) stated that chitosan-based materials induce several defensive genes in plants, such as pathogens-related genes like glucanase and chitinase. It also induces many enzymes, such as superoxide dismutase, catalase, and peroxidase. According to them, chitosan could be used as a biostimulant to stimulate plant growth and abiotic stress tolerance and induce pathogen resistance.

According to Meng and Tian (2009), the preharvest application of *Cryptococcus laurentii* combined with a low chitosan concentration significantly reduced the natural decay of table grape berries stored at 0°C. Preharvest chitosan sprays decreased *Botrytis* bunch rot severity compared to control and fungicide treatment in cv. Chardonnay (Reglinski et al., 2010).

In the study of De Bona et al. (2021), chitosan was applied on grapevine leaves before *Botrytis cinerea* inoculation to prevent fungal infection. Thanks to its fungistatic and filmogenic properties, chitosan protected grapevine leaves against *B. cinerea*. The study indicated that chitosan could protect the grapevine against *B. cinerea* infection. Chitosan elicits various defense reactions in plants, such as the stimulation of phenylalanine ammonia-lyase, peroxidase, and lipoxygenase activities, as well as the accumulation of phytoalexins and pathogenesis-related proteins (Aziz et al., 2003; Trotel-Aziz et al., 2006).

Orzali et al. (2017) reported that chitosan could be an active molecule that finds many possible applications in agriculture to reduce or replace chemical pesticides that cause more harm to the environment. Chitosan may represent an innovative eco-friendly strategy for managing plant diseases, replacing copper, or reducing its use due to its various previously described properties. Besides its low-cost production, chitosan is a sustainable and environmentally friendly molecule thanks to its biological properties, such as non-toxicity, biocompatibility, and biodegradability.

In the study of El-kenawy (2017), 500 mg L^{-1} concentration of chitosan application increased yield, total leaf area, and chlorophyll content in Thompson Seedless grape cultivar. The study of Khalil and Eldin (2021) aimed to determine the morphological and physiological effects of chitosan foliar spray and three irrigation levels on grapevines grown in plastic containers. Chitosan sprays significantly improved plant tolerance to water deficit by enhancing grapevine morphology and physiology under deficit irrigation conditions.

The studies mentioned above demonstrated that in the future, chitosan as a biostimulant might prevent and control some important fungal diseases in the grapevine. Also, it provides confidence to consumers concerned about fungicide residues in berries and wines. Chitosan, as a biostimulant, has been evaluated as a good candidate for substituting products of copper frequently used by winegrowers since it is a natural compound safe for humans and the environment. It could also be a suitable and applicable material for controlling grapevine diseases by organic growers.

Inorganic substances

In viticulture, temperatures above 35°C and drought caused by sunburns and excessive sun exposure are among the most important problems that may occur under the threat of global warming. In traditional viticulture, training systems should be selected carefully to protect against the harmful effects of the conditions mentioned, and attention is paid to summer pruning, especially leaf removal (Winkler et al., 1974; Çelik et al., 1998; Çelik, 2007). In recent years, aluminum silicate (kaolin) and potassium silicate compounds have also been used effectively as biostimulants to prevent heat and drought stress in viticulture (Bowen et al., 1992; Wang et al., 2004; Mickelbart et al., 2006; Wang et al., 2010; Coniberti et al., 2013; Dinis et al., 2016a, b; Bernardo et al., 2018; Singh et al., 2020; Cantürk and Kunter, 2021).

It has been reported that the foliar application of kaolin, a natural radiation-reflecting inert clay mineral, covers the plants in the form of a white-colored film layer, like a natural cuticle, which prevents heat and drought stress by reflecting sunlight. It also protects grapevine and other fruit crops against diseases and pests (Bowen et al., 1992; Glenn, 2012; Denaxa et al., 2012; Conde et al., 2016). As stated by Laane (2018), foliar application of micronutrients and biostimulants is an important nutrition method today and, in some cases, is more effective than soil application. Using foliar sprays of silicon compounds is assumed to compensate for the low Si uptake of roots due to the low level of absorbable silicon in the soil and the relatively complex absorption process of Si. According to the authors, foliar silicate applications have been used to reduce biotic stress since the 1990s. It has been reported that foliar silicate applications reduce various pathogen infections, but their effects on growth or yield are limited.

Many authors have focused on developing environmentally friendly practices, like kaolin, to sustain yield and quality in a challenging climate by reducing leaf and fruit surface temperature (Glenn, 2012; Dinis et al., 2016b). Singh et al. (2020) investigated the effects of aluminum (5%) and potassium silicates (0.1% and 0.05%) on the biochemical composition of Portuguese grapes (*Vitis vinifera* L. cv. Touriga Nacional and Touriga Franca). It was observed that phenolic composition and anthocyanin content differed between treatments. However, in terms of qualitative parameters observed in the study, there were non-significant changes in both treatments.

Cantürk et al. (2019) aimed to determine the effects of 3% foliar kaolin-based particle film treatment on histological properties and anthocyanin accumulation in Beauty Seedless and Tekirdag Seedless table grape cultivars in two growing seasons. According to the results, kaolin treatment increased total anthocyanin content and color intensity in both cultivars. In another study by Cantürk et al. (2018), it was investigated the effects of 3% kaolin and 4% dicarboxylic acid application on the volatile composition of the abovementioned grape cultivars. The results showed that the aromatic composition was not statistically affected in both cultivars. On the other hand, improvements in cluster, berry and skin color characteristics of cv. Trakya Ilkeren was reported via kaolin particle film (3%) treatment (Cantürk and Kunter, 2021).

Hosseinabad and Khadivi (2019) investigated the effects of four different concentrations (0, 2.5, 5 and 7.5%) of foliar aluminum silicate application on sunburn on cv Thompson Seedless in mid-June and mid-July. The results showed that the sunburn on the berries and leaves was lower in the treated vines than in untreated vines. The best result was achieved through kaolin treatment in mid-June, which could completely control sunburn on leaves. Kaolin treatment also positively affected cluster length, weight, density, berry length, and width.

Tacoli et al. (2019) showed that aluminum silicate, *B. thuringiensis*, and bunch-zone leaf removal treatments significantly reduced damage of the berry moth. Conde et al. (2016) evaluated the effects of foliar application of 5% aluminum silicate on fruit quality in Portugal. For this purpose, aluminum silicate was applied to the vines in July (in veraison). They stated that the aluminum silicate treatment reduced summer stress and affected fruit quality positively.

Song et al. (2012) investigated the effect of deficit irrigation and aluminum silicate (particle film) on berry components and volatile compounds in Merlot cultivar. Particle film was applied to leaves weekly from fruit set to harvest 60 g L^{-1} doses. It was determined that particle film application had a minimum effect on free and bound volatile compounds in grapes but increased the total anthocyanins.

Bowen et al. (1992) studied the effects of root or leaf applications of Si on powdery mildew severity in potted grapevines (*Vitis vinifera* L.). The foliar Si spray (pH=5.5) was applied one day ago inoculation with powdery mildew conidia. Foliar sprays at 17 mM Si substantially reduced the number of mildew colonies developed on inoculated leaves. Scanning electron micrographs showed that, on Si-sprayed leaves, hyphae did not develop in areas where thick Si layers were present on the leaf surface.

In the study of Al-Wasfy (2014), the effects of some treatments on berry weight and skin color in cv Flame seedless were determined. The treatments were potassium silicate at 250 ppm either singly or in various combinations with vitamins K, E, D, A, B_{12}, each at 50 ppm, and vitamin C at 500 ppm. The best results were obtained by treating the vines four times with a mixture of vitamins K, E, D, A, and B_{12}, each at 20 ppm, ascorbic acid at 500 pm, and silicon at 250 ppm.

In the study of Ramteke et al. (2012), Silixol (1, 2, and 3 ml L^{-1}) was sprayed twice (15 and 30 days after pruning), thrice (15, 30, and 45 DAP), and four times (15, 30, 45 and 60 DAP). The data on the benefit/cost ratio indicated that the application of Silixol twice 1 ml L^{-1} is economical for improving berry quality and yield of Thompson Seedless grapes.

Foliar kaolin applications of 50 gL^{-1} concentration in Touriga Nacional and Sauvignon Blanc cultivars reduced leaf and berry temperature. Increased ABA and IAA levels, improved water use efficiency, stomatal conductance, and photosynthesis, affected changes in antioxidant activity, improved sucrose synthesis and photoassimilate transport capacity in leaves, decreased DNA methylation and stimulated anthocyanins accumulation (Coniberti et al., 2013; Dinis et al., 2016a, 2017 and 2018; Bernardo et al., 2017; Conde et al., 2018). In the study of Dinis et al. (2016a), kaolin treatment on Touriga

Nacional grape cultivar increased total phenols (40%), flavonoids (24%), anthocyanins (32%), vitamin C (12%) and antioxidant capacity of the grape berries than control.

Beneficial fungi and bacteria

Microbial biostimulants include mycorrhizal and non-mycorrhizal fungi, bacterial endosymbionts (like Rhizobium), Plant Growth-Promoting Rhizobacteria (PGPRs), and Plant Growth Promoting Bacteria (PGPB) (Du Jardin, 2015; López-Bucio et al., 2015). Thus, microorganisms applied to plants can have a dual function of biocontrol agent and biostimulant, and the claimed agricultural effects will be instrumental in their regulatory categorization. Biofertilizers are biological products containing living microorganisms that, when applied to seed, plant surfaces, or soil, promote growth by several mechanisms such as increasing root biomass or root area, nutrient uptake capacity of the plant (Xiang et al., 2012; Calvo et al., 2014). Compared with chemical fertilizers, biofertilizers are environmentally friendly. They prevent damaging natural sources, help cleanse the plant from precipitated chemical fertilizers, and build healthy soil.

Plant growth-promoting rhizobacteria (PGPR) are naturally occurring soil bacteria that colonize plant roots and promote plant growth. They may help increase crop productivity by increasing biological nitrogen fixation availability or uptake of nutrients through phosphate solubility, siderophore production, biological fixation, and phytohormone production (El-Boray et al., 2013; El- Boray, 2021; Turan et al., 2021).

According to Ruzzi and Aroca (2015), to overcome the challenge of increasing food production with a significant reduction of agrochemical use and environmental pollution and an increase in natural resource productivity, the use of soil microorganisms in horticulture is essential. A group of microorganisms consisting of plant growth-promoting rhizobacteria (PGPR) has been studied since the beginning of the twentieth century, and their effects at the physiological level are currently well understood. PGPR mechanisms include hormone release or hormonal changes within plants, the production of volatile organic compounds, the improvement in nutrient availability, and the enhancement of abiotic stress tolerance.

According to Rouphael et al. (2015), to meet the increasing global food demand, an environmentally friendly way is to use the biostimulant functions of arbuscular mycorrhizal fungi (AMF). AMF supports plant nutrition by absorbing and translocating mineral nutrients (as biofertilizers) and induces changes in secondary metabolism leading to improved nutraceutical compounds. In addition, AMF interferes with the phytohormone balance of host plants, thereby influencing plant development (as bioregulators) and inducing tolerance to soil and environmental stress (as bioprotector). Maximum benefits from AMF activity have been achieved by adopting good farming practices (e.g., reduction of chemical fertilizers and biocides), inoculating efficient AMF strains, and the appropriate selection of plant host/fungus combinations.

Crupi et al. (2021) examined the effect of inactivated yeast (*Saccharomyces cerevisiae*) treatment before harvest on the anthocyanin content and berry quality of Scarlotta Seedless, Red Globe, and Crimson Seedless table grapes (*Vitis vinifera* L.). The yeast treatment enhanced the anthocyanin content in grapes. The increase in treated grapes has been kept after cold storage, too. The Chemical quality parameters of grapes were unaffected by the yeast treatment.

Hormone-containing substances (seaweed extracts and botanicals)

According to Bernardo et al. (2018) and Monteiro et al. (2022), exogenous application of some phytohormones such as salicylic acid, jasmonic acid, ABA, etc. signaling molecules, elicitors such as methyl jasmonate, yeast extracts, etc. has been found to help alleviate the heat stress in plants.

Salicylic acid (SA): Salicylic acid (SA), which takes its name from the Latin word Salix (willow), was first used by Rafacle Piria in 1838. SA, also regarded as a plant growth regulator or phytohormone, constitutes a group of phenolic substances. It has been determined that salicylic acid significantly affects plants grown under heavy metal, salinity, high temperature, and drought stress in the exogenous application (Hayat et al., 2010; El-kenawy, 2017; Altıncı et al., 2020; Blanch et al., 2020). Monteiro et al. (2022) stated that the exogenous application of SA would be an interesting agricultural application in the future to obtain quality grapes. However, it has also been noted that the effect may depend on several factors, including genotype, stress type/level, and applied concentration. Exogenous SA treatments increased total phenolic content and antioxidant activity in cv. Syrah and delayed ripening in cvs. Bez El Naka and Shiraz (Kraeva et al., 1998; Abdel Salam, 2016b; Blanch et al., 2020). In the experiment of Blanch et al. (2020), the effect of SA on the phenolic and antioxidant activity of *Vitis vinifera* cv. Shiraz was investigated. The treatments were carried out at the veraison, 8 to 9 weeks after full bloom. Total phenol content and the free radical scavenging activity increased via SA application of 100 mg L^{-1}. In particular, the total phenol content increased from 768.3 to 1843.5 mg 100 g^{-1}. Therefore, 100 mg L^{-1} was selected as the best SA concentration.

Abass et al. (2012) investigated the effect of spraying nutrient solution at four concentrations (0, 2, 3, 4) mL L^{-1} and SA at four concentrations (0, 50, 75, 100) mL L^{-1} on Halawani grape cultivar. They showed that SA and nutrient solution increased the total leaf area, total leaf chlorophyll concentration, total carbohydrates, total nitrogen, and C/N ratio. A combination treatment of 100 mg L^{-1} SA and 4 mL L^{-1} nutrient solution gave the best results compared to other combinations.

In the study of Abdel Salam (2016b), micronutrient mixture (Fe, Zn, Mn, and Br) at a concentration of 50 and 100 mg L^{-1} in a chelated form and SA at a concentration of 100 and 150 mg L^{-1} were applied in ‘Bez El Naka’ grapevine cultivar. The results proved that all parameters (cluster weight, berries weight, total chlorophyll content, NPK of leaves, TSS, acidity, total phenols, and β-carotene) were improved by all treatments compared to the control. Furthermore, treatments containing SA delayed ripening by about two weeks compared to the other treatments and control.

Kılınç Selek (2019) treated salicylic acid and 5-chlorosalicylic acid as chemical elicitors in Syrah grapevines. It was determined that both elicitors had various effects on physical (berry and cluster) and chemical properties. 0,75 mM doses of salicylic acid were determined as the best result in terms of phenolic compounds. Oraei et al. (2019) also stated that the total phenolic content increased significantly in SA-treated grapes (0.0, 50.0, 100.0, and 200.0 mM) compared to untreated grapes. In the study of El-Kareem and El-Rahman (2013), treating Ruby Seedless grapevines three times with a mixture containing roselle extract at 0.2 %, salicylic acid at 100 ppm, and seaweed extract at 0.2 % promoted yield and greatly decreased shot berries.

In their study to determine the thermal resistance of ^{14}C-salicylic acid in two-month-old "Jingxiu" grapevines (*Vitis vinifera* L.), Wang et al. (2004), 6th leaves of plants were treated with 35 µl ^{14}C-SA in the greenhouse environment. After then, 4th, 6th and 8th leaves were exposed to a high temperature of 38°C for 2, 6, or 12 hours, while other parts of plants were kept at 27°C. In control, all leaves have remained at 27°C for 2, 6, or 12 hours. As a result, SA accumulated in leaves exposed to heat stress, and immune signaling of heat shock proteins in the chloroplast was increased in both SA-treated and control leaves during heat stress. Researchers indicated that SA could be transported to a long distance and might be involved in the induction of heat tolerance.

Methyl jasmonate: Methyl jasmonate (MeJA) is reported to be a plant signal compound and a jasmonic acid (JA) derivative primarily isolated from the essential oil of *Jasminum grandiflorum* (Ju et al., 2016). MeJA is one of the most used elicitors in the grapevine and can induce defense mechanisms (Gutiérrez-Gamboa et al., 2019). Some studies have also reported that exogenous application of MeJA could improve berry and wine quality. Therefore, it is thought that the application of MeJA may be a simple and innovative strategy to improve the physicochemical and physiological parameters of vines and increase berry and wine quality (Monteiro et al., 2022)

In the study of Portu et al. (2015), foliar application of MeJA to Tempranillo grapevines increased anthocyanin and stilbene content in both grape and wine, besides enhanced wine flavonol content. According to study, the foliar application of MeJA could be a simple and accessible practice to improve grape and wine quality. In another study by Portu et al. (2016), MeJA, chitosan, and a commercial yeast extract were applied to the canopy on veraison and one week later veraison. Results showed that foliar treatments of

MeJA and yeast extract increased anthocyanin content. The yeast extract also increased grape stilbene content.

Gil-Muñoz et al. (2017a) also indicated that foliar benzothiadiazole and MeJA spray in Monastrell, Merlot, and Syrah grape cultivars could be useful to enhance the phenolic content of grapes and wines and to improve quality. In another experiment by Gil-Muñoz et al. (2017b), preharvest treatments of MeJA increased stilbenes in grapes and wines of cv. Monastrell and Tempranillo. Garde-Cerdán et al. (2022) concluded that the MeJA doped nanoparticle application to Tempranillo also increased some phenolic compounds and reduced the environmental stress.

Abscisic acid: Abscisic acid (ABA) is an elicitor extracted from plants. It is used as a growth regulator in vines and Its role is generally related to the abiotic stress response of vines (Ju et al., 2016; Gutierrez-Gamboa, 2019; Monteiro et al., 2022). It was reported that ABA reduced transpiration in Shiraz (Degaris et al., 2017), inhibited shoot growth, induced endodormancy, and improved berry color in Cabernet Franc and Chambourcin cultivars (Zhang, 2011). ABA protects plants from environmental stress and has a positive regulatory effect on plant growth. ABA stimulates fruit-level responses to abiotic stress, particularly related to water deficiency, light, and temperature, causing the accumulation of secondary metabolites in berry skin, pulp, and seeds as a defensive response against cell damage (Ferrandino and Lovisolo, 2014; Ju et al., 2016).

Seaweed extracts and others: Seaweed is a biostimulant of particular interest because of its availability globally. It was reported that brown seaweed (*Ascophyllum* spp.) improves plant growth, productivity, hormonal signaling, and secondary plant metabolism (Samuels et al., 2022). Brown seaweeds (*Ascophyllum* spp.) are widely used as liquid fertilizers in horticultural plants thanks to their plant-growth-promoting effects. They also have beneficial effects, including increasing yield and quality, longer shelf life, reducing fungal and insect attacks, and high plant tolerance to abiotic stresses such as salinity, extreme temperatures, nutrient deficiency, and drought (Kök et al., 2010). The great benefits of seaweed extract have been attributed to its chemical composition, which mainly consists of macro and microelements, antioxidants, natural hormones (cytokinins, auxins, and abscisic acid), vitamins, fatty acids, amino acids, and organic acids (Aziz et al., 2003; Battacharyya et al., 2015; Monteiro et al., 2022).

Non-aromatic vine-shoot extracts (Airén) have been recently proposed as "viticultural biostimulants" (Sánchez-Gómez et al., 2017). The foliar application of extracts from non-toasted (MVS) and toasted (MVSToasted) vine-shoots from aromatic cultivars (Moscatel) were made on Airén cultivar. There was an increase in grape yield but a lower alcohol degree in wines. The phenolic acids content of wine was affected positively overall by MVS.

Some studies on *Vitis vinifera* have shown that foliar application is beneficial for controlling and preventing the impacts of biotic and abiotic stress (Monteiro et al., 2022). Samuels et al. (2022) analyzed seaweed extracts in their work titled "Towards a Better Understanding of the Potential Benefits of Seaweed-Based Biostimulants in *Vitis vinifera* L. Cultivar." It has been reported that seaweed extracts could accelerate secondary metabolic pathways in the plant by triggering internal mechanisms. Turan and Köse (2004) determined the effect of seaweed extract on macro and micronutrient uptake of one-year-old grapevine (*Vitis vinifera* cv. Karaerik). Three seaweed extracts (Maxicrop, Proton, and Algipower) were sprayed on the leaves at different concentrations (0, 0.5, 1.0, and 2.0 g L^{-1}). Seaweed extract was more effective in supporting Cu uptake in vines than the nutrient element level of growth media. *A. nodosum* commercial extract increased Cu uptake and K+ and Ca^{2+} influx in grapevine, probably by increased cell membrane permeability. Besides, Sabir et al. (2014) reported that seaweed extract enhanced the leaf Zn and chlorophyll content. Irani et al. (2021) found that seaweed-treated grapevines had higher concentrations of N, P, K, Fe, and Zn than untreated vines under well-watered conditions. *A. nodosum* extract has increased berry size, weight, and firmness (Mancuso et al., 2006). Norrie et al. (2001) and Holden et al. (2008) also reported that *A. nodosum* seaweed extract has increased yield and ensured uniform maturation. Phenolic content increased by 1.063 mg cm^2 with the application of 1.5 kg ha^{-1} of seaweed extract from *A. nodosum* to Sangiovese berries, while the phenolic content increased by 0.951 mg cm^2 while *A. nodosum* algae extract was applied at a dose of 3 kg ha^{-1} (Frioni et al., 2018). The phenol content of the control was 0.753 mg cm^2 and increased grape yield with uniform ripening (Norrie et al., 2001; Holden et al., 2008).

Bioactive compounds present in seaweed extracts enhance plant performance under abiotic stress. Sprays of seaweed extracts have been shown to improve cold tolerance (Mancuso et al., 2006). In particular, the application of *A. nodosum* extracts improved freezing tolerance in grapes. The extract reduced the osmotic potential of the leaves, a key indicator of osmotic tolerance (Wilson, 2001). Kök and Bal (2016) showed that foliar application of seaweed and humic acid at 1000 ppm concentration improved some quality characteristics of cv. Riesling. In the study of Aziz et al. (2003), the -1,3-glucan laminarin derived from the brown algae *Laminaria digitata* was shown to be an efficient elicitor of defense responses in grapevine cells and effectively reduce *B. cinerea* and *P. viticola* development. Laminarin reduced *B. cinerea* and *P. viticola* infection by approximately 55% and 75%, respectively. The study indicated that activating defense responses using elicitors could be a valuable environmentally friendly strategy to protect plants against pathogens in sustainable viticulture. Arioli et al. (2021) revealed that the application of seaweed extract derived from *D. potatorum* and *A. nodosum* to the soil increased the yield by an average of 14.7% over consecutive years in Chardonnay, Semillon, Merlot, Cabernet Sauvignon cultivars.

3. CONCLUSION

The effects of climate change due to global warming are becoming more noticeable in viticulture as in many other fields. As a result, the biotic and abiotic stress negatively affects the growth and development of the vines, also grape yield and quality. For this reason, to reduce the effects of stress factors on the grapevine, the effective use of biostimulants was aimed to explain in this review. In addition to triggering the resistance and response mechanisms of plants to stress, the positive effects of biostimulants on nutrition have been shown. Therefore, using biostimulants is nowadays considered very important to reduce the use of synthetic fertilizers and pesticides. In this regard, it would be beneficial to continue studying different biostimulants in various grape cultivars grown in various soil and climatic conditions. In new studies, it should be prioritized to determine the biostimulant application method (from the leaves and soil), time, and concentrations that do not cause a decrease in grape yield, quality, and nutritional values. These efforts could ensure that grapes and grape products become increasingly safe and sustainable regarding the environment and human health. Because of all this, biostimulants and biofertilizers are considered a new environmentally friendly approach to increasing plant tolerance to biotic and abiotic stresses for organic and sustainable viticulture is considered very valuable.

REFERENCES

Abass, M.S.A., Zeen-Elabedeen, A.H.A. (2012). Effect of spraying nutrient solution and salicylic acid on vegetative growth characteristics of Halawani grape variety (*V.vinifera* L.). *Kufa J. Agric. Sci.* 4(1):65-80.

Abdel-Salam MM (2016a) Effect of foliar application with humic acid and two antioxidants on Ruby Seedless grapevine. *Middle East Journal of Agriculture Research* 5(2): 123–131.

Abdel-Salam, M.M. (2016b). Effect of foliar application of salicylic acid and micronutrients on the berries quality of "Bez El Naka" local grape cultivar. *Middle East Journal of Applied Sciences* 6(1): 178-188.

Alston, J. M., Sambucci, O. (2019). Grapes in the world economy. *The grape genome. Springer, Cham.*

Altıncı, N.T., Cangi, R. Üstün, D. (2020). Determining the effects of salicylic acid applications on high temperature stress in Narince grape variety. *Turkish Journal of Agriculture and Food Science and Technology* 8(5): 1227-1231.

Al-Wasfy, M.M.M. (2014). The synergistic effects of using silicon with some vitamins on growth and fruiting of Flame Seedless grapevines. *Stem Cells* 5: 8–13.

Arioli, T., Mattner, S. W., Hepworth, G., McClintock, D., McClinock, R. (2021). Effect of seaweed extract application on wine grape yield in Australia. *Journal of Applied Phycology* 33(3): 1883-1891.

Aziz, A., Poinssot, B., Daire, X., Adrian, M., Bézier, A., Lambert, B., ..., Pugin, A. (2003). Laminarin elicits defense responses in grapevine and induces protection against *Botrytis cinerea* and *Plasmopara viticola*. *Molecular Plant-Microbe Interaction* 16(12): 1118-1128.

Bassiony, S.S., El-Aziz, A., Maha, H., Fahmy, H.M. (2018). Effect of foliar application of putrescine and salicylic acid on yield, fruit quality and storability of Flame Seedless grape (*V.vinifera* L.). *Journal of Plant Production* 9(12): 1203-1214.

Battacharyya, D., Babgohari, M. Z., Rathor, P., Prithiviraj, B. (2015). Seaweed extracts as biostimulants in horticulture. *Scientia Horticulturae* 196: 39-48.

Belal, B.E.A. (2015). Effect of some biostimulants of growth, yield and berry quality of King's Ruby grapevines. *Egyptian Journal of Horticulture* 42(1): 135-152.

Bernardo, S., Dinis, L.T., Luzio, A., Pinto, G., Meijón, M., Valledor, L., Conde, A., Gerós, H., Correia, C.M., Moutinho-Pereira, J. (2017). Kaolin particle film application lowers oxidative damage and DNA methylation on grapevine (*Vitis vinifera* L.). *Environmental and Experimental Botany* 139: 39–47.

Bernardo, S., Dinis, L.T., Machado, N., Moutinho-Pereira, J. (2018). Grapevine abiotic stress assessment and search for sustainable adaptation strategies in Mediterranean-like climates. A review. *Agronomy for Sustainable Development* 38(6): 1-20.

Blanch, G.P.; Gómez-Jiménez, M.C.; del Castillo, M.L.R. (2020). Exogenous salicylic acid improves phenolic content and antioxidant activity in table grapes. *Plant Foods for Human Nutrition* 75: 177–183.

Bowen, P., Menzies, J., Ehret, D., Samuels, L., Glass, A.D. (1992). Soluble silicon sprays inhibit powdery mildew development on grape leaves. *Journal of the American Society for Horticultural Science* 117(6): 906-912.

Calvo, P., Nelson, L., Kloepper, J.W., (2014). Agricultural uses of plant biostimulants. *Plant Soil* 383: 3–41.

Canellas, L.P., Olivares, F.L., Aguiar, N.O., Jones, D. L., Nebbioso, A., Mazzei, P., Piccolo, A. (2015). Humic and fulvic acids as biostimulants in horticulture. *Scientia Horticulturae* 196: 15-27.

Cantürk, S., Kunter, B., Coksari, G. (2018). Effects of kaolin and dicarboxylic acid based stress inhibitors on aroma composition of two table grape cultivars (*V.vinifera* L.). *Acta Scientarum Polonorum Hortorum Cultus* 17(5): 37-46

Cantürk, S., Kunter, B., Buyukkartal, N. (2019). Effects of kaolin particle film on berry histological properties in two table grape cultivars (*V.vinifera* L.). *Journal of Berry Research* 9(2): 309-319.

Cantürk, S., Kunter, B. (2021). Effects of kaolin treatment on table grape characteristics of cv. Trakya Ilkeren (*V. vinifera* L.). *KSU Journal of Agriculture and Nature* 24(3): 522-528.

Champa, W.H., Gill, M.I.S., Mahajan, B.V.C., Bedi, S. (2015). Exogenous treatment of spermine to maintain quality and extend postharvest life of table grapes (*V.vinifera* L.) cv. Flame Seedless under low temperature storage. *LWT-Food Science and Technology* 60(1): 412-419.

Chen, Y., Clapp, C.E., Magen, H. (2004). Mechanisms of plant growth stimulation by humic substances: The role of organo-iron complexes. *Soil Science and Plant Nutrition* 50(7): 1089-1095.

Colla, G., Rouphael, Y. (2015). Biostimulants in horticulture. *Scientia Horticulturae* 196: 1-2.

Colla, G., Nardi, S., Cardarelli, M., Ertani, A., Lucini, L., Canaguier, R., Rouphael, Y. (2015). Protein hydrolysates as biostimulants in horticulture. *Scientia Horticulturae* 196: 28-38.

Conde, A., Pimentel, D., Neves, A., Dinis, L.T., Bernardo, S., Correia, C. M., ..., Moutinho-Pereira, J. (2016). Kaolin foliar application has a stimulatory effect on phenylpropanoid and flavonoid pathways in grape berries. *Frontiers in Plant Science 7: 1150.*

Conde, A., Neves, A., Breia, R., Pimentel, D., Dinis, L.T., Bernardo, S., Correia, C.M., Cunha, A., Gerós, H., Moutinho-Pereira, J. (2018). Kaolin particle film application stimulates photoassimilate synthesis and modifies the primary metabolome of grape leaves. *Journal of Plant Physiology* 223: 47–56.

Coniberti, A., Ferrari, V., Dellacassa, E., Boido, E., Carrau. F., Gepp V, Disegna, E. (2013) Kaolin over sun-exposed fruit affects berry temperature, must composition and wine sensory attributes of Sauvignon Blanc. *European Journal of Agronomy* 50: 75–81.

Crupi, P., Palattella, D., Corbo, F., Clodoveo, M.L., Masi, G., Caputo, A.R., ..., Tarricone, L. (2021). Effect of pre-harvest inactivated yeast treatment on the anthocyanin content and quality of table grapes. *Food Chemistry* 337: 128006.

Çelik, S. (2007). Bağcılık (Ampelololoji). Cilt 1. Trakya Üniversitesi Tekirdağ Ziraat Fakültesi Bahçe Bitkileri Bölümü, 1. Avcı Ofset., İstanbul.

Çelik, H., Ağaoğlu, Y.S., Fidan, Y., Marasalı, B., Söylemezoğlu, G. (1998). Genel Bağcılık. Sunfidan AŞ Mesleki Kitaplar Serisi 1. Fersa Matbaacılık, Ankara.

De Bona, G.S., Vincenzi, S., De Marchi, F., Angelini, E., Bertazzon, N. (2021). Chitosan induces delayed grapevine defense mechanisms and protects grapevine against *Botrytis cinerea*. *Journal of Plant Diseases and Protection* 128(3): 715-724.

Degaris, K.A., Walker, R.R., Loveys, B.R., Tyerman, S.D. (2017). Exogenous application of abscisic acid to root systems of grapevines with or without salinity influences water relations and ion allocation. *Australian Journal of Grape and Wine Research* 23(1): 66–76.

Denaxa, N. K., Roussos, P. A., Damvakaris, T., Stournaras, V. (2012). Comparative effects of exogenous glycine betaine, kaolin clay particles and Ambiol on photosynthesis, leaf sclerophylly indexes and heat load of olive cv. Chondrolia Chalkidikis under drought. *Scientia Horticulturae* 13: 87-94.

De Orduna, R.M. (2010). Climate change associated effects on grape and wine quality and production. *Food Research International* 43(7): 1844-1855.

Dinis, L.T., Bernardo, S., Conde, A., Pimentel, D., Ferreira, H., Félix, L., Gerós, H., Correia, C.M., Moutinho-Pereira, J. (2016a). Kaolin exogenous application boosts antioxidant capacity and phenolic content in berries and leaves of grapevine under summer stress. *Journal of Plant Physiology* 191: 45–53.

Dinis, L.T., Ferreira, H., Pinto, G., Bernardo, S., Correia, C.M., Moutinho-Pereira, J. (2016b). Kaolin-based, foliar reflective film protects photosystem II structure and function in grapevine leaves exposed to heat and high solar radiation. *Photosynthetica* 54(1): 47–55.

Dinis, L.T., Malheiro, A.C., Luzio, A., Fraga, H., Ferreira, H., Gonçalves, I., Pinto, G., Correia, C.M., Moutinho-Pereira, J. (2017). Improvement of grapevine physiology and yield under summer stress by kaolin-foliar application: water relations, photosynthesis and oxidative damage. *Photosynthetica* 56: 641–651.

Dinis, L.T., Bernardo, S., Luzio, A., Pinto, G., Meijón, M., Pintó-Marijuan, M., Cotado, A., Correia, C., Moutinho-Pereira, J. (2018). Kaolin modulates ABA and IAA dynamics and physiology of grapevine under Mediterranean summer stress. *Journal of Plant Physiology* 220: 181–192.

Du Jardin, P. (2012). The science of plant biostimulants–A bibliographic analysis, Ad hoc study. report. https://orbi.uliege.be/bitstream/2268/169257/1/Plant_ Biostimulants _final_report_bio_2012_en.pdf.

Du Jardin, P. (2015). Plant biostimulants: Definition, concept, main categories and regulation. *Scientia Horticulturae* 196: 3-14.

EBIC, (2022). Economic overview of the biostimulants sector in Europe. European Biostimulants Industry Council. https://biostimulants.eu/highlights/economic-overview-of-the-european-biostimulants-market/ and https://biostimulants.eu/ issue/plant-biostimulants-contribute-to-climate-smart-agriculture/

El-Boray, M.S.S., Mostafa, M.F.M., Hamza, D.M. (2013). Effect of humic acid, bio-fertilizers and mıcro-elements on leaf mineral contents of Kings Ruby grapevines. *Journal of Plant Production* 4(6): 871-883.

El-Boray, M. (2021). Effect of humic acid, bio-fertilizers and some micro-elements on leaf mineral contents of King Ruby grapevines. *Academia Letters* Article 661.

El-kenawy, M. A. (2017). Effect of chitosan, salicylic acid and fulvic acid on vegetative growth, yield and fruit quality of Thompson Seedless grapevines. *Egyptian Journal of Horticulture* 44(1): 45-59.

El-Kareem, G.M., El-Rahman, A.M. (2013). Response of Ruby Seedless grapevines to foliar application of seaweed extract, salicylic acid and roselle extract. *Hortsci J Suez Canal Univ*. 1: 294-303.

Ferrandino, A., Lovisolo, C. (2014). Abiotic stress effects on grapevine (*Vitis vinifera* L.): Focus on abscisic acid-mediated consequences on secondary metabolism and berry quality. *Environmental and Experimental Botany* 103: 138-147.

Ferrara, G., Brunetti, G. (2010). Effects of the times of application of a soil humic acid on berry quality of table grape (*Vitis vinifera* L.) cv Italia. *Spanish Journal of Agricultural Research* 8(3): 817-822.

Frioni, T., Sabbatini, P., Tombesi, S.; Norrie, J., Poni, S., Gatti, M., Palliotti, A. (2018). Effects of a biostimulant derived from the brown seaweed *Ascophyllum nodosum* on ripening dynamics and fruit quality of grapevines. *Scientia Horticulturae* 232: 97–106.

Garde-Cerdán, T., de Urturi, I.S., Rubio-Bretón, P., Marín-San Román, S., Baroja, E., Ramírez-Rodríguez, G. B.,..., Pérez-Álvarez, E.P. (2022). Foliar application of methyl jasmonate and methyl jasmonate supported on nanoparticles: Incidence on grape phenolic composition over two seasons. *Food Chemistry* 134244.

Gil-Muñoz, R., Bautista-Ortín, A.B., Ruiz-García, Y., Fernández-Fernández, J.I., Gómez-Plaza, E. (2017a). Improving phenolic and chromatic characteristics of Monastrell, Merlot and Syrah wines by using methyl jasmonate and benzothiadiazole. *OENO One* 51: 17–27.

Gil-Muñoz, R., Fernández-Fernández, J.I., Crespo-Villegas, O., Garde-Cerdán, T. (2017b). Elicitors used as a tool to increase stilbenes in grapes and wines. *Food Research International* 98: 34–39.

Glenn, D.M. (2012). The mechanisms of plant stress mitigation by kaolin-based particle films and applications in horticultural and agricultural crops. *Hort Science* 47(6): 710-711.

Gutiérrez-Gamboa, G, Portu, J, López, R, Santamaría, P, Garde-Cerdán, T. (2018). Elicitor and nitrogen applications to Garnacha, Graciano and Tempranillo vines: effect on grape amino acid composition. *Journal of Science of Food and Agriculture* 98(6): 2341-2349.

Gutiérrez-Gamboa, G., Romanazzi, G., Garde-Cerdán, T., Pérez-Álvarez, E.P. (2019). A review of the use of biostimulants in the vineyard for improved grape and wine quality: Effects on prevention of grapevine diseases. *Journal of Science of Food and Agriculture* 99: 1001-1009.

Halpern, M., Bar-Tal, A., Ofek, M., Minz, D., Muller, T., Yermiyahu, U. (2015). The use of biostimulants for enhancing nutrient uptake. *Advances in Agronomy* 130: 141–174.

Hayat, Q., Hayat, S., Irfan, M., Ahmad, A. (2010). Effect of exogenous salicylic acid under changing environment: A review. *Environmental and Experimental Botany* 68: 14–25.

Hayes, M. A., Shor, A. C., Jesse, A., Miller, C., Kennedy, J. P., Feller, I. (2020). The role of glycine betaine in range expansions; protecting mangroves against extreme freeze events. *Journal of Ecology* 108(1): 61-69.

Holden, D., Johnson, H., Ocafrain, M., Norrie, J., Fidelibus, M. (2008). Effect of seaweed extract on fruit set, yield, and quality in Pinot Noir winegrapes. In Proc. 35th A. Meet. *Plant Growth Regulation Society of America. 3-7.*

Hosseinabad, A., Khadivi, A. (2019). Foliar application of kaolin reduces the incidence of sunburn in Thompson Seedless grapevine. *European Journal of Horticulture and Science* 84(3): 171-176.

Hussain Wani, S., Brajendra Singh, N., Haribhushan, A., Iqbal Mir, J. (2013). Compatible solute engineering in plants for abiotic stress tolerance-role of glycine betaine. *Current Genomics* 14(3): 157-165.

Irani, H., ValizadehKaji, B., Naeini, M.R. (2021). Biostimulant-induced drought tolerance in grapevine is associated with physiological and biochemical changes. *Chemical and Biological Technologies in Agriculture* 8(1): 1-13.

Jalil, O.T.J. (2017). *The effects of glycine betaine applications on the development of vines grown under limited irrigation conditions*. (Doctoral thesis) Selcuk University, Konya.

Ju, Y.L.; Liu, M.; Zhao, H.; Meng, J.F.; Fang, Y.L. (2016). Effect of exogenous abscisic acid and methyl jasmonate on anthocyanin composition, fatty acids, and volatile compounds of Cabernet Sauvignon (*Vitis vinifera* L.) grape berries. *Molecules* 21: 1354.

Kauffman, G.L., Kneivel, D.P., Watschke, T.L. (2007). Effects of a biostimulant on the heat tolerance associated with photosynthetic capacity, membrane thermostability, and polyphenol production of perennial ryegrass. *Crop Science* 47: 261–267.

Khalil, H.A., Eldin, R.M.B. (2021). Chitosan improves morphological and physiological attributes of grapevines under deficit irrigation conditions. *Journal of Horticultural Research* 29(1): 9-22.

Kılınç Selek, N. (2019). *Farklı dönemlerde uygulanan salisilik asit ve 5-klorosalisilik asit elisitörlerinin syrah üzüm çeşidinde kalite özellikleri üzerine etkileri* (Master's thesis). Namık Kemal University. Tekirdağ.

Kök, D., Bal, E., Celik, S., Ozer, C., Karauz, A. (2010). The influences of different seaweed doses on table quality characteristics of cv. Trakya Ilkeren (*V. vinifera* L.). *Bulgarian Journal of Agriculture and Science* 16(4): 429-435.

Kök, D., Bal, E. (2016). Effects of foliar seaweed and humic acid treatments on monoterpene profile and biochemical properties of cv. Riesling berry (*V. vinifera* L.) throughout the maturation period. *Journal of Tekirdag Agricultural Faculty* 13(2): 67.

Kraeva, E., Andary, C., Carbonneau, A., Deloire, A. (1998). Salicylic acid treatment of grape berries retards ripening. *Vitis* 37: 143–144.

Külahtaş, B., Çokuysal, B. (2016). Biyostimulantların sınıflandırılması ve Türkiye'deki durumu. *Çukurova Tarım ve Gıda Bilimleri Dergisi* 31(3): 185-200.

Laane, H. M. (2018). The effects of foliar sprays with different silicon compounds. *Plants* 7(2): 45.

López-Bucio, J., Pelagio-Flores, R., Herrera-Estrella, A. (2015). Trichoderma as biostimulant: exploiting the multi level properties of a plant beneficial fungus. *Scientia Horticulturae* 196: 109-123.

Mancuso, S., Azzarello, E., Mugnai, S., Briand, X. (2006). Marine bioactive substances (IPA extract) improve foliar ion uptake and water stress tolerance in potted *Vitis vinifera* plants. *Advances in Horticultural Science* 20: 156–161.

Meng, X., Tian, S. (2009). Effects of preharvest application of antagonistic yeast combined with chitosan on decay and quality of harvested table grape fruit. *Journal of the Science of Food and Agriculture* 89: 1838–1842.

Meng, J.F., Yu, Y., Shi, T.C., Fu, Y.S., Zhao, T., Zhang, Z.W. (2019). Melatonin treatment of pre-veraison grape berries modifies phenolic components and antioxidant activity of grapes and wine. *Journal of Food Science and Technology* 39(1): 35-42

Mickelbart, M.V., Chapman, P., Collier-Christian, L. (2006). Endogenous levels and exogenous application of glycine betaine to grapevines. *Scientia Horticulturae* 111(1): 7-16.

Mirdehghan, S.H. Rahimi, S. (2016). Pre-harvest application of polyamines enhances antioxidants and table grape (*Vitis vinifera* L.) quality during postharvest period. *Food Chemistry* 196: 1040-1047.

Monteiro, E., Gonçalves, B., Cortez, I., Castro, I. (2022). The role of biostimulants as alleviators of biotic and abiotic stresses in grapevine: A review. *Plants* 11(3): 396.

Mostafa, M.F.M., EL-Boray, M.S., El-Baz, E.L., Omar, A.S. (2017). Effect of fulvic acid and some nutrient elements on King Ruby grapevines growth, yield and chemical properties of Berries. *Journal of Plant Production* 8(2): 321-328.

Norrie, J., Branson, T., Keathley, P.E. (2001). Marine plant extracts impact on grape yield and quality. *Int. Symp. Fol. Nutr. Perenn. Fruit Plants* 594: 315–319.

OIV, (2019). 2019 Statistical report on world vitiviniculture. International Organisation of vine and wine intergovernmental organisation. https://www.oiv.int/public/medias/6782/oiv-2019-statistical-report-on worldvitiviniculture.pdf

Oraei, M., Panahirad, S., Zaare-Nahandi, F., Gohari, G. (2019). Pre-véraison treatment of salicylic acid to enhance anthocyanin content of grape (*V. vinifera* L.) berries. *Journal of Science of Food and Agriculture* 99(13): 5946-5952.

Orzali, L., Corsi, B., Forni, C., Riccioni, L. (2017). Chitosan in agriculture: a new challenge for managing plant disease. In: Biological activities and application of marine polysaccharides, Intech Open.

Paradikovic, N., Vinkovic, T., Vrcek, I.V., Zuntar, I., Bojic, M., Medic-Saric, M. (2011). Effect of natural biostimulants on yield and nutritional quality: an example of sweet yellow pepper (*Capsicum annuum* L.) plants. *Journal of Science of Food and Agriculture* 91: 2146–2152.

Pichyangkura, R., Chadchawan, S. (2015). Biostimulant activity of chitosan in horticulture. *Scientia Horticulturae* 196: 49-65.

Popescu, G.C. (2019). Biostimulants and agri-environment measures in order to increase the agricultural sustainability. *International Symposium on Advanced Engineering Technologies. ISADET,* 16s.

Portu, J., Santamaría, P., López-Alfaro, I., López, R., Garde-Cerdan, T. (2015). Methyl jasmonate foliar application to Tempranillo vineyard improved grape and wine phenolic content. *Journal of Agricultural and Food Chemistry* 63(8): 2328-2337.

Portu, J., López, R., Baroja, E., Santamaría, P., Garde-Cerdán, T. (2016). Improvement of grape and wine phenolic content by foliar application to grapevine of three different elicitors: Methyl jasmonate, chitosan, and yeast extract. *Food Chemistry* 201: 213–221.

Ramteke, S.D., Kor, R.J., Bhanga, M.A., Khot, A.P., Zende, N.A., Datir, S.S., Ahire, K.D. (2012). Physiological studies on effects of silixol on quality and yield in Thompson Seedless grapes. *Ann. Plant Physiol* 26: 47–51.

Reglinski, T., Elmer, P.A.G., Taylor, J.T., Wood, P.N., Hoyte, S.M. (2010). Inhibition of *Botrytis cinerea* growth and suppression of botrytis bunch rot in grapes using chitosan. *Plant Pathology* 59(5): 882-890.

Rienth, M., Vigneron, N., Darriet, P., Sweetman, C., Burbidge, C., Bonghi, C., ..., Castellarin, S.D. (2021). Grape berry secondary metabolites and their modulation by abiotic factors in a climate change scenario–a review. *Frontiers in Plant Science* 12: 643258.

Rouphael, Y., Colla, G. (2020). Biostimulants in agriculture. *Frontiers in Plant Science* 11(40): 1-7.

Rouphael, Y., Franken, P., Schneider, C., Schwarz, D., Giovannetti, M., Agnolucci, M., ..., Colla, G. (2015). Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops. *Sci. Hort*. 196: 91-108.

Ruzzi, M., Aroca, R. (2015). Plant growth-promoting rhizobacteria act as biostimulants in horticulture. *Scientia Horticulturae* 196: 124-134.

Sabir, A., Yazar, K., Sabir, F., Kara, Z., Yazici, M.A., Goksu, N. (2014). Vine growth, yield, berry quality attributes and leaf nutrient content of grapevines as influenced by seaweed extract (*Ascophyllum nodosum*) and nanosize fertilizer pulverizations. *Scientia Horticulturae* 175: 1-8.

Samuels, L.J., Setati, M. E., Blancquaert, E.H. (2022). Towards a better understanding of the potential benefits of seaweed based biostimulants in *Vitis vinifera* L. cultivars. *Plants* 11(3): 348.

Sánchez-Gómez, R., Zalacain, A., Pardo, F., Alonso, G.L., Salinas, M.R. (2017). Moscatel vine-shoot extracts as a grapevine biostimulant to enhance wine quality. *Food Research. International* 98: 40–49.

Savvas, D., Ntatsi, G. (2015). Biostimulant activity of silicon in horticulture. *Scientia Horticulturae* 196: 66-81.

Searchinger, T., Hanson, C., Ranganathan, J., Lipinski, B., Waite, R., Winterbottom, R., ..., Heimlich, R. (2013). The great balancing act. Working Paper, Installment 1 of Creating a Sustainable Food Future. Washington, DC: World Resources Institute.

Singh, R.K., Afonso, J., Nogueira, M., Oliveira, A.A., Cosme, F., Falco, V. (2020). Silicates of potassium and aluminium (kaolin); comparative foliar mitigation treatments and biochemical insight on grape berry quality in *Vitis vinifera* L. (cv. Touriga National and Touriga Franca). *Biology* 9(3): 58.

Song, J., Shellie, K.C., Wang, H., Qian, M.C. (2012). Influence of deficit irrigation and kaolin particle film on grape composition and volatile compounds in Merlot grape (*V.vinifera* L.). *Food Chemistry* 134(2): 841-850.

Tacoli, F., Cargnus, E., Moosavi, F.K., Zandigiacomo, P., Pavan, F. (2019). Efficacy and mode of action of kaolin and its interaction with bunch-zone leaf removal against *Lobesia botrana* on grapevines. *Journal of Pest Science* 92(2): 465-475.

Tilman, D., Balzer, C., Hill, J., Befort, B.L. (2011). Global food demand and the sustainable intensification of agriculture. *Proceedings of the national academy of sciences* 108(50): 20260-20264.

Trotel-Aziz, P., Couderchet, M., Vernet, G., Aziz, A. (2006). Chitosan stimulates defense reactions in grapevine leaves and inhibits development of *Botrytis cinerea*. *European Journal of. Plant Pathology* 114(4): 405-413.

Turan, M., Köse, C. (2004). Seaweed extracts improve copper uptake of grapevine. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science* 54(4): 213-220.

Turan, M., Yildirim, E., Ekinci, M. Argin, S. (2021). Effect of biostimulants on yield and quality of cherry tomatoes grown in fertile and stressed soils. *HortScience* 56(4): 414-423.

Wang, L.J., Huang, W.D., Zhan, J.C., Yu, F.Y. (2004). The transport of 14 C salicylic acid in heat-stressed young *Vitis vinifera* plants. *Russian Journal of Plant Physiology* 51(2): 194-197.

Wang, L.J., Fan, L., Loescher, W., Duan, W., Liu, G.J., Cheng, J.S., ... , Li, S.H. (2010). Salicylic acid alleviates decreases in photosynthesis under heat stress and accelerates recovery in grapevine leaves. *BMC plant Biology* 10(1): 1-10.

Wilson, S. (2001). Frost management in cool climate vineyards. *Final Report to Grape and Wine Research & Development Corporation.*

Winkler, A.J., Cook, J.A., Kliewer, W.M., Lider, L.A. (1974). General Viticulture. *University of California press. Berkeley, Los Angeles, London.*

Xiang, W., Zhao, L., Xu, X., Qin, Y., Yu, G. (2012). Mutual information flow between beneficial microorganisms and the roots of host plants determined the bio-functions of biofertilizers. *American Journal of Plant Sciences* 3: 1115–1120.

Zamani, M.M., Rabiyei, V., Nejatian, M.A. (2013). Effect of proline and glycine betaine application on some physiological characteristics in grapevine under drought stress. *Iranian Journal of Horticultural Science* 43(4): 393-401.

Zhang, Y. (2011). Foliar application of abscisic acid induces dormancy responses in greenhouse-grown grapevines. *HortScience* 46(9): 1271–1277.

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