

#### ORGANIC FARMING IN MANY WAYS

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(The Licence Number of Publicator: 2014/31220)

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**ISBN:** 978-625-8405-00-2 February / 2022 Ankara / Turkey Size = 16x24 cm

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#### **PREFACE**

Today, when we begin to feel the negative effects of the global climate crisis intensely, we better understand how important it is to do agriculture with the right techniques. It is now known that the damages caused by the intensive synthetic inputs applied in the agricultural production process to the soil, water, air and all living things, especially the negative effects of nitrogen and pesticides on global warming, cannot be underestimated.

In addition, the pandemic process caused by Covid-19 at the beginning of 2020 reminded people once again the importance of healthy eating and keeping their immune system strong. The way to a healthy and correct diet is through herbal and animal products that are growed in a natural and clean environment and do not contain intense preservatives or additives.

In plant and animal productions made using organic farming techniques, production is carried out in a way that does not harm the environment and living things without using synthetic inputs. The fact that every stage from the field to the table is controlled increases its reliability. While people reach healthy and high quality foods, the use of agricultural inputs that cause global warming is taken under control, preventing environmental pollution. Instead of using external inputs in closed system production, natural substances and nutrient cycle are used. Soil fertility, water resources, biodiversity are protected and their sustainability is ensured.

In this context, studies and researches to contribute to plant and animal organic production continue without slowing down. In our book, there are microbial fertilizations and soil improvers, organic fertilizations, the use of organic-containing preparations for plant protection, the place and importance of medicinal and aromatic plants in organic agriculture, and scientific studies and compilations on organic plant production in recent years.

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## **CHAPTER 1**

# FERTILIZERS FOR ORGANIC FARMING: A NEW PERSPECTIVES

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#### INTRODUCTION

Proper nutrition are essential for high yields and quality crop products. Organic farming systems are mostly nitrogen limited. Organic nitrogen fertilizer sources are differ from synthetic nitrogen fertilizers in isotopic composition. Green manure crop incorporation in soils, legume including crop rotations and farmyard manure are major nitrogen sources supplying other nutrients, too. But alternative fertilisers are gaining interest with increasing sustainable agriculture tagets. Local system-oriented approaches may get help from diversified organic fertilizer sources such as legume crop pellets, animal by-products, aquatic plant products, blood meal, bone meal, corn gluten, crab/crustacean meal, feather meal, fermentation products, fish meal food processing by-products, hoof and horn meal, insect frass, kelp meal, molasses, peanut meal, oyster shell lime, plant extracts, seaweed, sawdust, shellfish meal, soybean meal, sugar, wool, worm castings etc.

To meet the demand for food, farmers use synthetic fertilizers enormously. Although this technique helped many countries to increase crop yields, many problematic issues exist. Synthetic fertilizers increased the cost of agricultural production, decreased soil fertility, degradate local ecosystems by released pollutants in environment. Farmers need alternatives eliminating pollution problems but increasing crop yields. Organic fertilizers or biofertilizers are cost-effective eco-friendly alternatives for improving soil fertility and quality parallel with protecting ecosystem (Baweja et al., 2019).

During 1970s, criticism on intensive agriculture and chemical industry favoured the growth of organic agriculture. Organic farming at 1970s was a rebellious movement. In 1980s with a orientation in policy, the organic movement get official recognition (Monnier, 2004). Despite increasing demand on organic products everyday, research on organic farming (genotype selection, fertilizers etc.) are limited (Huang et al., 2016).

Organic farming systems are mostly nitrogen limited (Fortune et al., 2004). Organic nitrogen fertilizer sources are differ from synthetic nitrogen fertilizers in isotopic composition (Chalk et al., 2014). Nitrogen fertilization in organic farming during plant growth is very problematic. Well adopted crop rotations and proper nutrition are essential for high yields and quality crop products. Organic fertilizers and green manure crop incorporation in soils are good options in organic farming (Rosae, 2018).

Primary input in organic farming for soil fertility source from grass, legumes and other plants. These are get ploughed into soil as green manure. Other major source is farmyard manure. Minor part sources from fertilizers or composted / fermented house waste. Many organic farmers use livestock to produce fertilizers. Especially for cereals and vegetable crops, need of animal manure is quite large due to high export of nutrients from soils (Eriksen & Kristensen, 2004). During last years, organic farming has grown exponentially worldwide. As a result, animal manure usage to improve soil fertility has become main choice. But usage of manure as fertilizer may increase steroid hormone

metabolites transfer to soils (Verderame et al., 2016). Organic farming is based on system-oriented approaches (Kumar et al., 2019). Alternative fertilisers are gaining interest with increasing sustainable agriculture tagets (Oliver et al., 1997).

Organic farming fertilizer types are highly diversified. Following product groups are allowed for use in accordance with National Organic Program standards of USDA: Activated charcoal, alfalfa meal or pellets, amino acids, anaerobic digestate, animal by-products, aquatic plant products, ash (plant or animal), bark, basalt, biochar, blood meal, bone meal, clay, cocoa bean hulls, coconut fiber, compost tea, compost (plant and animal materials), corn gluten, crab/crustacean meal, diatomaceous earth, dolomite (mined), enzymes, feather meal, fermentation products, fish meal and powder, fish products, food processing by-products, fulvic acids, greensand (glauconite), gypsum (mined source), homeopathic preparations, hoof and horn meal, humates, humic acids, insect frass, kelp meal, limestone, magnesium rock, manure, meat meal, microbial inoculants, meat by-products and waste, microbial products, mined minerals (unprocessed), molasses, mulch, peanut meal, peat moss, mycorrhizae, neem and neem derivatives, oyster shell lime, perlite, phosphate rock, plant extracts, plant preparations, plants, sea salt, seaweed and seaweed products, pulverized rock, pumice, sawdust, shellfish meal, soybean meal, sphagnum moss, sugar, vermiculite, wool, worm castings, yucca, zeolite (OMRI, 2021).



Fig. 1. Industrial pellet mill for biomass pelleting (Dien et al., 2018)



Fig. 2. Raw material to be processed into crustacean meal (Anonymus, 2021)



Fig. 3. Bone meal crushed for 75 μm (200 mesh) (Payus et al., 2014)

## 1. FEATHER MEAL

Huge amounts of feathers (175.000 tonnes/year) are produced from chicken process (5-6% of chicken weight) in Europe but limited number of companies produce feather meal. Its resistant structured keratin is needed higher temperature-pressure-time to be transformed into feather meal compared to meat and bone meal. Generally, feather meal contains 70-80% crude protein and has a digestibility of 60-70%. Prices are between 250-550 €/tones (Shuliy & Løes, 2014).



Fig 3. Chicken feathers are wastes of broiler chicken processing (Acda, 2010)

Feather meal contains 15% nitrogen as insoluble keratin which is an organic nitrogen fertilizer (Hadas & Kautsky, 1994).

#### 2. BLOOD MEAL

For prevention of iron chlorosis which is a widespread agronomical problem, blood meal is a fertilizers allowed in organic farming (Yunta et al., 2013). Blood meal provides nitrogen with iron bound to porphyrin organic compounds as a fertilizer in organic farming (Yunta et al., 2013).

Meat and bone meal recycling back to agricultural lands in past was common as a major source of organic nitrogen and phosphorus nutrients. But after the Bovine Spongiform Encephalopathy crisis in 1999, although bone and meat meals are allowed by EU in organic farming, many grower organisations banned them after that crisis (Möller, 2015).

# 3. AQUATIC MACROPHYTES, SEAWEEDS (MACROALGAE)

Deficiencies of phosphorus due to soil fixation, run-off, erosion exports by crops is a major problem in farming systems. Excess phosphorus of farming systems generally ends up in waters, result with eutrophication and growth of aquatic plants. Aquatic macrophytes can recovering phosphorus from sediments and waters. Aquatic plant biomass production for the phosphorus exist in waters and sediments is needed to be assessed in near future (Stabenau et al., 2018).

Usage of seaweeds and its extracts in agriculture to improve plant growth and stress tolerance was common for centuries. Beneficial effects of seaweeds source from phytohormone contents (Wally et al., 2013).

Seaweed extracts is a biostimulant category. Biostimulants are gaining increased attention for crop management and to increase crop yields. *Ascophyllum nodosum* extracts are widely used for this aim. (Goni et al., 2016; Khan et al., 2011). Reason for fast emergence of biostimulants as crop management products is mainly their capacity to enhance productivity of crops under abiotic stress conditions (Goni et al., 2018).



Fig. 4. Canopy of macroalgae Ascophyllum nodosum (top) and Fucus serratus (bottom) (Photo: Galice Hoarau) (Jueterbock et al., 2013).

Seaweed extracts are recently a sustainable tools to combat abiotic stress (Salvi et al., 2019). Brown seaweeds have several advantageous when their their crude extracts are sprayed on plants. Unlike chemical fertilizer, these biofertilizers are non-toxic, biodegradable, nonhazardous to humans and animals and non-polluting (Fatimah et al., 2018).

#### 4. WOOL MEAL

Waste wool is a problem in Europe. Because crossbred sheeps are not selected for fine wool and as a result, their wool are coarse and containing many dead fibres. These wools are a by-product which is mostly disposed off. Superheated water hydrolysis is a new technology to transform waste wool into fertilizers. Wool keratin (protein) degragates into simple compounds ready to be released (Zoccola et al., 2015).

#### 5. PANCHAGAVYA

"Panchagavya" is produced by mixing cow urine, cow dung, cow ghee, cow milk and cow curd. It is promoting growth and strengthen immunity of plant against pest and diseases. It contagins vitamins, nitrogen, phosphorus, micronutrients and various amino acids (Ram, 2017).

#### 6. SEEDCAKES

Application of processed agro-industrial by-products to soils in organic farming improve soil fertility especially for short cropping cycled vegetables (Montemurro et al., 2015). Processing oil crops to produce biodiesel generate seedcake as wastes. This product can improve soil properties (Eroa, 2015).

#### CONCLUSIONS

Novel processing techniques suitable to organic fertilizer production are increasing in number everyday. Continuous scan of these methods is a requirement to reduce costs of organic farming especially in developing coutries like Turkey. By this way consumption of healthy organic products may become more widespred. As organic farmers diversify and mix their fertilizer types, they may benefit more properties (antimicrobial, biostimulnt, micronutrient source properties etc.) of these different types

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# **CHAPTER 2**

# THE EFFECT OF VERMICOMPOST APPLICATIONS ON SOIL PHYSICAL AND CHEMICAL PROPERTIES IN AGRICULTURAL PRODUCTION

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#### INTRODUCTION

Although it is considered to increase the productivity of the areas used in agricultural production, maintaining the current productivity levels should be one of the most basic goals. However, although unconscious fertilization and spraying increase soil fertility at the beginning, it returns in the form of negative effects in the later process (Ceylan et al., 2000). The key to soil fertility is its organic matter content. As a result of intensive tillage with agricultural production, significant decreases occur in the organic matter content of soils. Organic carbon content of our country's soils Sönmez et al. (2018) reported that because of the project they carried out, 70.5% were very few, 18.08% were in the middle class and 9.78% were in the middle class. However, according to the Eyüpoğlu (1999) publication, when the organic matter content of our country's soils is examined, it has been reported that 21.47% of our country's soils contain very little, 43.78% less and 22.62% moderate organic matter. It has been observed that significant losses in organic carbon/organic matter contents have occurred because of unconscious agricultural activities in areas containing medium and low organic matter in the period of approximately 19 years. The same is true for world lands. As a matter of fact, Lal (2004) reported that because of his research, agricultural soils lost 50-66% of their initial carbon. It has also been reported that approximately 10-15% of anthropogenic greenhouse gas emissions are caused by losses in agricultural soils (Smith et al., 2007). As a result of their research, Budak and Günal (2018) determined that the organic carbon stock (TOCS) of the soil was the highest in forest areas (44.33

Mg ha<sup>-1</sup>), and the lowest in the fields where field crops were planted (28.91 Mg ha<sup>-1</sup>). Even the result of this study shows us how weak the areas with intensive agriculture are in terms of organic carbon. This situation reveals how suitable the soils are for carbon storage. We will both bring the carbon that it has lost for a long time to the soil and contribute to reducing the rate of carbon in the atmosphere, which is the main actor in global warming. In this context, what needs to be done is to restore carbon resources, especially vegetable carbon resources, to the soil. With the activities of enriching the soils in terms of organic carbon source, significant benefits will be provided both in alleviating global warming and the continuation of the fertility of the soils. In this way, in addition to increasing the productivity of agricultural production lands, the protection of soils against erosion and the recovery of areas that have lost their productivity will be ensured. As a result of organic fertilizer applications, it is important in plant production with positive and important effects such as improving the soil structure, providing a good texture and structure, increasing the water and nutrient holding capacity by aeration, regulating the soil reaction, and increasing the number of beneficial soil microorganisms (Adiloğlu and Eraslan, 2012) increases will be made. To ensure the accumulation of organic C in agricultural production lands, the application of management systems that minimize soil tillage, maximize the amount of harvest waste on the land surface, and production systems that increase water and nutrient use efficiency (Paustian et al., 1997) should be encouraged.

Fertilizers are the main input of agricultural production. In addition, seed and seed supply, irrigation, agricultural spraying, labor costs and fuel costs come to the fore. Even if the chemical fertilization to be made depending on the soil analysis provides significant increases in agricultural production for the production season, organic fertilizers should be given weight in terms of soil fertility. Long-term chemical fertilizer applications also cause significant changes in the physical, chemical and biological structure of the soil. Depending on the climate and soil conditions, this change can be positive or negative. For example, long-term ammonium sulfate fertilizer applications in tea growing areas in our country have caused the soil pH of some places to fall below 4.5. On the other hand, organic fertilizer applications cause significant improvements in the physical, chemical, and biological properties of the soil.

The source of organic fertilizer applied to agricultural fields is usually barnyard manure, but such as green manure, municipal waste manure, sewage sludge, worm manure, raw or pellet chicken manure, solid and liquid humic acid originating from leonardite, and liquid humic acid of vegetable origin produced with the industrial investments made today. compost products are used. As organic fertilizers of animal origin, meat combination residues such as blood powder and meat residues, horn and nail powder, skin powder and bone powder are applied. At this point where technology has come, the use of byproducts, such as biochar, as well as hydrochar and pyrochar, which are released after electricity production obtained by gasification

technology from biosolids, which are renewable energy sources, is becoming widespread. These products (biochar, hydrochar and pyrochar) should be preferred to be used as soil conditioners, not with the intention of organic fertilizer.

As a new trend, the production and use of vermicompost has an important place today. Worms (Allolobophora caliginosa species), which are commonly found in the soil, are species that prefer to live in the soil even though they produce compost from organic materials (Demir et al., 2010) and cannot be used in the production of vermicompost from organic materials. Of the 3000 worm species found in the world, only California Red Worm Lumbricus rubellis and Eisenia foetida worm species are more preferred to be used in the production of vermicompost from organic materials (Julka, 1986). These earthworm species contain symbiotic bacteria (Rhizobium) and nitrogen-fixing bacteria from asymbiotic microorganisms (Azotobacter) and mycorrhizal fungi (Edwards, 1998), N-fixing and phosphate-solving bacteria by passing mixtures of animal origin organic fertilizers and plant materials through their digestive systems (Edwards, 1998; Chaulagain et al., 2017), containing humic acid and fulvic acid that promote plant growth (Demir et al., 2010), high porosity and cation exchange capacity, good aeration and water holding capacity, high microbial activity (Bossuyt et al., 2005; Tejada and González, 2009) and help the formation of a good organic fertilizer that has a long-lasting effect on the soil (Sing et al., 2014).

Vermicompost applications and ornamental plants (Boran, 2015), strawberries (Yaviç et al., 2020), corn (Durukan et al., 2020), potatoes (Yourtchi et al., 2013), red onions (Bai and Malakouti, 2007), curly lettuce (Hernandez et al. 2010; Hınıslı, 2014), tomato (Azarmi et al., 2008) and many other plants have been reported to positively affect product quantity and quality. This situation manifests itself in the form of significant increases in yield and yield criteria as a reflection of the improvements in the physical and chemical properties of the soils with the applications of vermicompost. That is, it is a result of the improvement of soil quality.

When soil quality is mentioned, soil pH, nutrient balance, water content, composition of soil atmosphere and biotic factors come to mind. These factors are directly affected by the applied organic fertilizers (Marinari et al., 2000). As a matter of fact, it has been reported in many studies that there are improvements in the physical, chemical and biological properties of soils with vermicompost applications (Ali et al. 2007; Rangarajan et al. 2008; Hernandez et al. 2010; Lazcanoa and Dominguez, 2011; Pathama and Sakthivel, 2012; Vanlı and Bedük, 2013; Tavalı et al., 2014; Rajkhowa et al., 2017). With vermicompost applications, the capacity of soils to absorb heavy metals and positively charged ions increases (Herwijnen et al., 2007; Bolan and Duraisamy, 2003). In this way, vermicompost can be used as a good organic fertilizer in the phytoremediation of soils. Angelova et al. (2010) investigated the mobility of Cu, Zn, Pb and Cd in the soil after increasing doses of compost, vermicompost and paet applications

to the growing medium. At the end of the study, they reported that the organic fertilizers applied decreased the available ligand of Cu, Zn, Pb and Cd compared to the control soil. With this benefit of vermicompost, a better plant development and cultivation can be done without the danger of heavy metals. Indeed, Wang et al. (2012) reported that phytoextraction of cadmium increased with the application of vermicompost to cadmium-contaminated soils, the residue of root and shoot dry matter of the plant grown, as well as the microbial population of soil samples and without any negative change in enzyme activity. In another study, it was reported that increasing vermicompost applications decreased the Co, Cd, Ni and Pb contents of cucumber (Adiloğlu et al., 2017). The worms obtained by accumulating some heavy metals in their own structures (Shahmansouri et al., 2005; Panday et al., 2013: Singh et al., 2014; Singh and Kalamhad, 2016; Swati and Hait, 2017) used in the production of applied vermicompost It should also be considered that the heavy metal content of the fertilizer may be at levels that do not pose a risk.

In this review, we evaluated the results of our study, and the effects of worm castings applied in increasing doses on some physical properties of the soil and macro element contents were examined.

#### MATERIAL AND METHOD

The study was carried out in the Central District of Karabük Province, Cemal Plain Locality, Karademir Ziraat Ltd. in the fall of 2017. Sti. carried out in the unheated plastic greenhouse in the treatment farm. Soil samples used in the study were taken from a depth of 0-20 cm from the farmland, and after they became air-dried, they were sieved through a 2 mm sieve and filled into pots with 5 kg of soil. Worm manure was mixed at the rates of 0%, 2.5%, 5%, 10% and 20% according to the weight calculation. The experiment was planned according to the randomized plot design with 3 replications and 5 pots in each replication. In addition, to compare with chemical fertilizer application, ammonium sulfate (21% N), triple super phosphate (TSP, 44% P<sub>2</sub>O<sub>5</sub>) and potassium sulfate as 75 mg N kg<sup>-1</sup>, 50 mg P<sub>2</sub>O<sub>5</sub> kg<sup>-1</sup> 75 mg K<sub>2</sub>O kg<sup>-1</sup>. (50% K<sub>2</sub>O) fertilizers were weighed and added to the experiment as a separate subject. A total of six subjects were formed in the study and it was carried out with a total of 90 flowerpots. The study started on 13.09.2017 and ended on 05.11.2017. The experiment continued for 53 days and at the end of this period, soil samples were duly taken and analyzed.

As routine analyzes in the soil sample taken for the trial; With the Bouyoucous method (Bouyoucous, 1951), pH in the soil samples applied at the end of the trial; According to a 1:2.5 soil:water suspension (Jackson, 1958), organic matter; modified Walkey Black method (Walkey, 1947), salt analysis; According to Richards (Richards, 1954), total nitrogen; by the micro Kjeldahl method (Kacar,

1994), available phosphorus; Sodium bicarbonate with blue color method (Olsen et al., 1954), exchangeable potassium, calcium, magnesium and sodium: 1 N with ammonium acetate method (Thomas, 1982). Exchangeable cations contents of the obtained extracts were determined in Inductively Coupled Plasma Mass Spectroscopy (ICP-MS, Thermo Scientific X Series) device. The obtained data were analyzed with CoStat statistical programs, and the important data were lettered according to Duncan's multiple comparison test (Düzgüneş et al. 1987).

**Table 1.** Some physical and chemical analysis results of soil and vermicompost used in the study

			EC	Lime	O.M.	Nitrogen	P	K
	Texture	pН	dS		%	_	mg kg <sup>-1</sup>	
			m <sup>-1</sup>					
Soil	Clay-loamy	7.41	0.67	13.2	2.73	0.16	7.7	385
Vermicompost	-	6.90	3.7	-	20	1.20	10900	35100

#### **RESULTS**

The variance analysis results of the effects of the applications on the pH, salt, lime, organic matter, nitrogen, phosphorus, potassium, and sodium contents of the soil are given in Tables 2. The averages of the physical and chemical properties and the effects of macro elements are given in Figures 1 and 2.

While the effects of the applications on the pH, salt, lime, organic matter, nitrogen, phosphorus, potassium, sodium, contents of the soil were significant at the P<0.001 level, the effects on the calcium content was determined to be insignificant (Table 2).

**Table 1.** Variance analysis table of the effects of applications on soil lime, organic matter, pH and salt contents with macro elements contents

Source	D.f.	L	ime	Organic matter		pН		Salinity	
		M.S.	F	K.O	F	K.O	F	K.O	F
Treatments	5	2.0601	15.8 **	57.752	34.2 **	0.4029	75.9**	5669985	86.5 **
Error	12	0.1305		1.687		0.0053		65493	
Source	D.f.	Nit	rogen	Phosphorus		Potassium		Calcium	
		M.S.	F	K.O	F	K.O	F	K.O	F
Treatments		0.1401	387.9 **	61.892	1547.0 **	1714521	31.2**	3351	0.13 ns
Error		0.0003		0.004		54884		24489	
Source	D.f.	Sodium							
		M.S.	F	-					
Treatments		166860	21.76**	-					
Error		7668							

<sup>\*\*, %1;</sup> ns, non-significant

While increasing vermicompost applications to the experimental environment caused decreases in pH and lime contents of the soil, it caused an increase in salt and organic matter contents. The pH, which was 8.16 in the control, decreased to 7.27 with 20% vermicompost application. This decrease was realized as 12.2%. While the lime content was 15.96% in the control, it decreased to 13.86% with 20% vermicompost application, and this decrease was 15.15%. The salt content of the trial soils increased with increasing vermicompost applications. The salt content, which was 860 µS cm<sup>-1</sup> in the control, increased to 2988 µS cm<sup>-1</sup> with 20% vermicompost application. This increase was 247.4%. A similar situation was also detected in soil organic matter content. The organic matter content, which was 2.71% in the control, increased to 13.61% with 20% vermicompost application, and this increase was 402.2% (Figure 1).

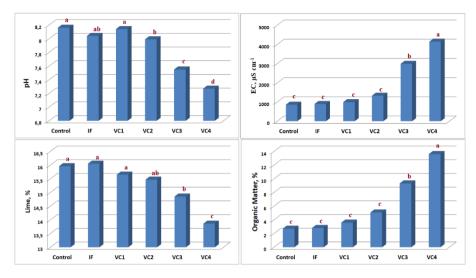
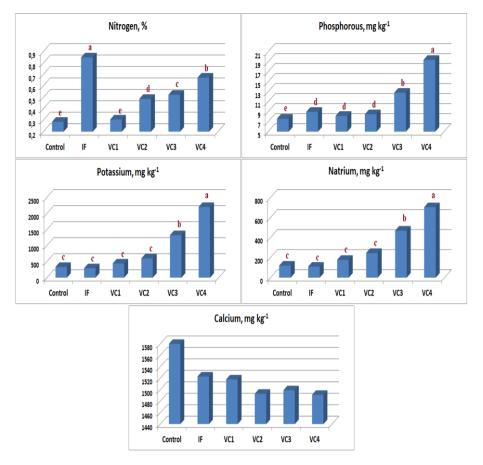


Figure 1. Values of the effects of applications on soil pH, salt, lime, and organic matter contents and Ducan lettering, IF; Inorganic Fertilizer, VC1; 2.5%, VC2; 5.0% VC3; 10%, VC4; 20%.

Applications increased the nitrogen, phosphorus, potassium, and sodium contents of the soil. The values of respectively 0.287%, 7.60 mg kg<sup>-1</sup>, 336.3 mg kg<sup>-1</sup> and 119.8 mg kg<sup>-1</sup> in the control increased with increasing vermicompost applications and the highest values were 0.673%, 19.37 mg kg<sup>-1</sup>, 2204.3 mg kg<sup>-1</sup> and 703.7 mg kg<sup>-1</sup> in 20% vermicompost application, respectively. These increases were 134.5%, 154.9%, 555.5% and 487.4%, respectively. Calcium content decreased in 20% VC application compared to control, but this decrease was not statistically significant. The actual decrease was 5.9% (Figure 2).



**Figure 2.** Values of the effects of applications on total nitrogen, available phosphorus and available potassium, calcium, and natrium contents of the soil and Ducan lettering, IF; Inorganic Fertilizer, VC1; 2.5%, VC2; 5.0% VC3; 10%, VC4; 20%

## DISCUSSION AND CONCLUSION

It has been reported in many studies that vermicompost applications have significant effects on the physical, chemical, and biological properties of the soil (Atiyeh et al., 2000; Ntanos and Koutroubas, 2002; Çıtak et al., 2011; Bellitürk et al., 2015). In this study, it was

determined that there were significant changes in the physical and chemical values of the test soil with vermicompost applications. In addition, it has been determined that soils cause significant increases in the available heavy metal content. The increases in salt and organic matter contents of the trial soils are probably related to the high salt and organic matter contents of vermicompost (Table 1). When compared to the salinity classes determined for the soils (Kacar, 1994), the salinity values measured in soil samples with high-dose vermicompost applications (10 and 20% VC) are slightly salty (2-4 mS cm<sup>-1</sup>) and moderately salty (4-8 mS cm<sup>-1</sup>). It was determined that he took place in the classes. In this case, the appropriate dose should be recommended considering the physical properties of the soil and the salt sensitivity of the plant variety to be grown in vermicompost applications.

It was determined that there was a statistically insignificant decrease in the lime content of the soils with vermicompost applications (Figure 1). These decreases increased as the applied vermicompost dose increased. This may be since the amount of soil taken from the sample with 20% vermicompost applied during the lime analysis was proportionally less than the control soils.

It was determined that there were significant decreases in pH values of soil samples after the application (Figure 1). This is since the pH value of the vermicompost is within neutral pH values (Table 1), as well as the binding area of high amount of H<sup>+</sup> ions of organic matter and causing changes in soil pH (McCauley et al., 2017). As a matter of fact, because of their research, Ritchie and Dolling (1985) reported that H+ ions released by organic matter had a significant effect on soil pH.

After the application, the total nitrogen content of the analyzed soils increased with vermicompost application compared to the control (Figure 2). It has also been reported in similar studies that there is an increase in the total nitrogen content with vermicompost applications (Ntanos and Koutroubas, 2002). With vermicompost applications, it increases the decomposition of organic materials in the soil with the beneficial organisms it contains (Gopal et al., 2009; Pathma and Sakthivel, 2012) and the increase in the beneficial organism population in the soils it is applied to (Sinha et al., 2010). increases the nitrogen content. It is reported that long-term vermicompost applications increase the nitrogen holding capacity of the soil (Hepperly et al., 2009).

Although agricultural areas contain organic inorganic phosphorus in certain proportions, the usefulness of this phosphorus to the plant is limited. Benefit of the plant decreases with the fixation it creates with calcium in alkaline reaction soils and with iron and aluminum in acid reaction soils. Therefore, continuous phosphorus fertilization is needed. Vermicompost contains phosphate-soluble bacteria (PSB) (Alikhani et al., 2017) and increases the solubility of phosphorus, as well as malonic, fumaric and succinic acids (Epstein, 1997) released during the conversion of organic matter into vermicompost by worms, and soluble humic molecules (Atiyeh et al. 2002) helps the plant to

benefit more from phosphorus. Among the results of our study, it is seen that the available phosphorus content increased with increasing vermicompost applications (Figure 2).

While significant increases were obtained in the useful potassium content of vermicompost applied soils compared to the control and inorganic fertilizer applications, there was a slight decrease in the available calcium content (Figure 2). This may be due to the high amounts of potassium and sodium content of the vermicompost fertilizer. As a matter of fact, Özkan et al. (2016) reported that vermicompost applied in increasing amounts increased the available potassium content of the soil, but this increase was not statistically significant. Najafi-Ghiri (2014) reported that vermicompost applications provide significant increases in the water-soluble, extractable, and non-extractable potassium contents of the soil compared to the control, because of his research on the effect of zeolite and vermicompost applications on the potassium content of the soil. The enrichment of the soil with both potassium and other elements by vermicompost applications may have resulted from the conversion of some organic acids released during the decomposition of the vermicompost into the retrievable form of the elements in the non-absorbable form and the conversion of the elements in the organic form into the inorganic form by some microorganisms in the structure of the vermicompost. In addition, vermicompost contains enzymes such as amylase, lipase, cellulase, and chitinase, which continue to break down soil organic matter (to release nutrients and present it to

plant roots) even after disposal (Chaoui et al., 2003; Tiwari et al., 1989).

It was determined that the calcium content of the soil, which is available to plants, decreased with the increase in vermicompost applications (Figure 2). While it has been reported in general studies that vermicompost applications increase the extractable calcium content of the soil (Azarmi et al., 2008; Yan et al., 2013; Mahmud et al., 2018), it has been observed that there is a decrease in our findings. This situation could be caused by two reasons. First, vermicompost may be due to the absorption of extractable calcium by the soil with its high porosity, and secondly, due to the improvements in the physical properties of the soil with vermicompost applications, the plants growing in this soil can take up more calcium.

An increase in the useful sodium content was determined with vermicompost applications (Figure 2). This may be due to the high salt content of vermicompost (Table 1). Demir (2020) reported that 2.5% vermicompost application causes an increase in the extractable sodium content of the soil compared to the control, while 5% vermicompost application causes a decrease. It is also reported that there is a decrease in the extractable sodium content of the soil with vermicompost applications (Sandoval et al., 2015).

Vermicompost (worm castings) contains symbiotic, asymbiotic microorganisms, mycorrhizal fungi and actinomycetes (Edwards, 1998; Demir et al., 2010) as well as worm secretions, growth

hormones, enzymes, vitamins, and amino acids (Edwards and Bohlen 1996; Sinha et al. 2013) is a rich fertilizer material. With these beneficial properties, vermicompost suppresses diseases and pests (Arancon et al., 2004; Citak et al., 2011; Tutar, 2013), increases the beneficial organism population in the soil (Arancon et al., 2006), is used in plant cultivation. It provides positive changes in physical, chemical, and biological parameters (Parthasarathi et al., 2008; Atmaca, 2012) and provides significant improvements in plant growth and yield and nutrient intake (Ativeh et al., 2001; Edwards et al., 2004; Gutie'rrez-Miceli et al., 2007; Çıtak et al., 2011; Açıkbaş & Bellitürk, 2016).

As result, vermicompost applications provide significant improvements in the physical and chemical properties of soils. The issue to be considered is the salt content and heavy metal content of vermicomposts. In this context, it is necessary to carry out applications based on soil analysis so that the soils do not become saline and are not polluted by heavy metals.

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## **CHAPTER 3**

# INNOVATIVE MATERIAL APPROACH IN AGRICULTURAL PRODUCTION: ACTIVE DRY YEAST

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## INTRODUCTION

The nutritional requirement of the increasing population has led human beings to buy more products per unit area in agriculture, and over time, technologies and methods have been developed for this purpose (chemical fertilizers and pesticides, hybrid technology). These methods have resulted in a tremendous product increase, but unfortunately the environmental impacts in this process could not be measured. The excessive and unconscious use of these developed methods has led to toxicity in the soil, deterioration of the nutrient balance and adversely affecting the sustainability of the soils (Rojas et al., 2016). The results obtained from the studies conducted in the world in recent years, it is observed that the widespread and misuse of chemical-containing plant nutrition and plant protection products used in plant production has turned into an important global environmental and health problem, and this situation poses great threats in terms of sustainable agriculture and food safety. Similar studies conducted in our country have yielded results reflecting the same concerns (Tiryaki et al., 2010). These chemical fertilizers and pesticides do not only remain in the area where they are applied, but also spread over a wide area over time. It is stated that 70% to 99% of pesticides applied to plants are transported to different ecological environments through wind, rain and irrigation and reach non-target organisms in these environments (Tiryaki, 2016). In summary, as a result of the intensive use of chemical pesticides and especially nitrate fertilizers in plant production; Increasing pollution in soils, groundwater and atmosphere has become a serious threat to

human and animal health, wildlife and the environment around the World (Huang et al., 2019).

In recent years, researchers have conducted studies on the use of environmentally friendly materials of organic origin in order to eliminate the damage to human health and ecosystem as a result of the use of artificial and synthetic plant growth and development regulators. The nature-friendly materials used in these studies either promote the yield-fertilizer relationship by increasing fertilizer efficiency or assume the role of fully synthetic fertilizers. Rational use of organic and inorganic food sources; It is important in terms of reducing dependence on chemical fertilizers, minimizing nutrient losses, making sustainable production with high efficiency and increasing nutrient use efficiency to optimum levels. The integrated use of organic materials and chemical fertilizers can be one of the ways to achieve sustainable agriculture and environment (Ahmad et al., 2006). Organic materials are promising with their multi-nutrient content and more economical availability. However, the effects of these materials on soil and crops are long-term. Therefore, manufacturers are not very willing to use it. However, it may be more attractive to use with chemical fertilizers. In a study, the use of half of the recommended mineral nitrogen in wheat production and farm manure together had similar yield results with the use of full mineral N (Warraich et al., 2002). In addition, the use of effective microorganisms along with organic and inorganic fertilizers is an effective method in terms of promoting nutrient release. For example, increasing mycorrhizal activity in soils helps to alleviate the increased

need or deficiency for macro and micro nutrients. The ability of organic production depends on the presence of beneficial fungi and bacteria in the soil's natural microflora, such as rhizobacteria (PCPR), arbuscular mycorrhizal fungi (AMF), which promote plant growth and development. Biosimulants and biofertilizers; chemical fertilizers, plant protection products, are low-cost and environmentally friendly sources that can be an alternative to growth regulators (Kawalekar, 2013). Biofertilizers, when applied to seeds and soil, participate in the nutrient cycle and increase productivity (Sing et al., 2011). Studies have shown that 60-90% of the fertilizers applied in production are lost and only 10-40% of them can be used by plants. Therefore, microbial inoculants are of great importance in terms of environmental health and agricultural sustainability (Adesemoye & Kloepper, 2009). Biosimulants are natural substances or beneficial microorganisms used to improve the effective use of nutrients by plants, to increase resistance to stress conditions and to improve product quality (Du jardin, 2015).

The relationship of biosimulants and biofertilizers with the organic production system is increasing. The biggest problem in organic production is the decrease in the amount of product. With the researches done so far, it is possible to stop the decrease in the amount of product with the use of biosimulants. These products can be very useful in improving agricultural sustainability and provide reduced environmental impact and high yields (Ertani et al., 2015). Researchers continue to look for materials that can minimize environmental damage without reducing production. Active dry yeast is one of the materials

that is currently gaining momentum. Recently, dry yeast has been used as an alternative growth source in the bio-organic fertilization system. For this reason, it is necessary to focus on the role of yeasts in the system and their mechanism of action in order to promote sustainable production.

#### 1.USES OF YEAST

Yeasts were discovered by Dutch scientist Anton de Leeuwenkoek and English scientist Robert Hooke with the invention of the microscope, and their roles in the fermentation process were determined by Pasteur in 1937. Yeast has been used in making bread, wine and beer since ancient times. Yeasts are single-celled micro-fungi that can assimilate different sources of carbon and nitrogen. Because they are facultatively anaerobic, some also have the ability to ferment carbohydrates, with the main mode of reproduction being asexual, budding, or cell division (Kurtzman et al., 2011). The nutrients it needs for growth and development are carbohydrates (hexoses, pentoses), alcohols, and organic acids. It lacks hydrolytic enzymes to metabolize carbohydrates, sucrose, lactose, and maltose (Barnett, 1975). When their usage areas are examined, the most known yeasts worldwide with their applications in the food industry are in the biofuel production industry (Kwak et al., 2019), biomedical (Kumar et al., 2014) and the agricultural sector (Mukherjee et al., 2020) has also increased its applications in different fields. Because yeast can reproduce easily and feed widely, they exist in great quantities in the ecosystem (Yurkov, 2018). Also, they

contribute to remedy soil biologically by assimilating toxic compounds from pesticides, due to their adaptation to the habitat (Dai et al., 2010).

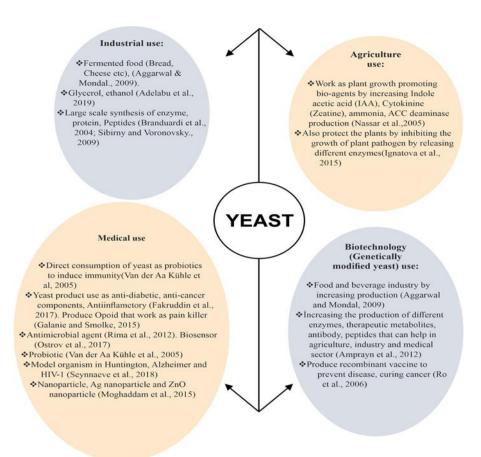
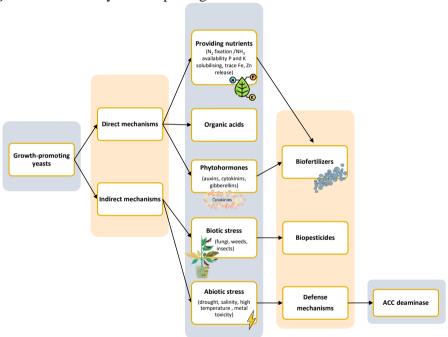


Figure 1. Sectors where yeast is used

Although the use of yeast in industry is well known, its use in agriculture is not yet widespread. Studies have been conducted on the use of bacteria, fungi and algae as growth promoters in agriculture (Mukherjee & Patel., 2019). The results of studies on the use of yeast in agricultural production have also begun to be reported. The literature

shows that yeast has a bio-agent feature that directly and/or indirectly helps to improve plant growth characteristics (Yu et al., 2008). The direct effect that promotes plant growth is related to the production of different hormones and enzymes. Plants need hormones such as IAA and stokinin during their development. In addition, the development of plants is related to the presence of macro and micro nutrients such as phosphorus, potassium, zinc, magnesium, manganese.

Figure 2. Effect of yeast on plant growth



It is known that about 1500 yeast species have been listed (Kurtzman et al., 2011). Saccharomyces cerevisiae, one of the yeast species, has taken an important place in industrial development. Saccharomyces cerevisiae, which is used in different industries such as bread industry, bioethanol production, chemical production, alcohol fermentation, has been evaluated from a different perspective today. Yeast is considered a remarkable new growth stimulator for different crops. Dry yeast can function in agricultural production as a nutritive, stimulant, preservative and useful biomaterial. When we look at the content of active dry yeast, it is seen that it is a natural source of many growth substances (thiamine, riboflavin, niacin, pyridoxine and vitamins B1, B2, B3 and B12), cytokinins and many nutrients, as well as protein, carbohydrates, lipids, nucleic acid and organic compounds (Nagodawithana, 1991). Tartoura (2001) In baker's yeast extract, carbohydrates, protein, plant growth regulators GA, IAA and necessary for plant growth N 1.2%, P 0.13%, K 0.03%, Br 0.016%, Zn 0.05% Na% He found that it contains elements such as 0.01, Mg 0.07%, Fe 0.13%, Ca 0.02%, in different proportions.

**Table 1.** The Composition of Active Dry Yeast.

Amino acids	Value (mg g <sup>-1</sup> )	Minerals	Value (mg g <sup>-1</sup> )	Vitamins	Value (mg g <sup>-1</sup> )
Tryptophan	0.45	P	12.5	Vit. B1	2.23
Valine	2.19	K	30	Vit. B2	3.33
Glutamic	2.00	Na	56	Vit. B6	1.25
Serine	1.59	Mg	2	Vit. B12	0.15
Aspartic	1.33	Ca	0.1	Thiamine	2.71
Cysteine	0.23	Mn	5.69	Riboflavin	4.96
Proline	1.53	Zn	69.5	Inositol	0.26
Tyrosine	2.09	Cu	0.02	Biotin	0.09
Arginine	1.99	Fe	0.05	Niacin	5.3
Histamine	2.31	Co	0.005	Cholin	0.04
Isoleocine	2.31	S	13.5		
Leucine	3.09	Si	0.03		
Lysine	2.95	Ni	0.003		
Methionine	0.72	Sn	0.003		
Phenylalanine	2.01	Cr	0.002		
Threonine	2.09				

Mahmood et al., 2020

Active dry yeast is considered a natural source of cytokinins and biostimulants that stimulate cell growth and division, chlorophyll formation, and nucleic acid and protein synthesis. Yeast extract; It increases biological activity, enzyme activity, photosynthetic pigments and vegetative growth and antioxidant content, metabolism and water holding capacity (Abbas, 2013), and also increases the rate of photosynthesis by releasing CO2 into the environment (Kurtzman et al., 2011). In addition to reducing the use of inorganic fertilizers, which are harmful to the environment, in agriculture, it increases the yield and fruit quality and ensures long-term storage of vegetables and fruits (Shaaban et al., 2015). The literature shows that yeast has a bio-agent feature that directly and/or indirectly helps to improve plant growth characteristics (Yu et al., 2008). The direct effect that promotes plant growth is related to the production of different hormones and enzymes. Plants need hormones such as IAA and stokinin during their development. In addition, the development of plants is related to the presence of macro and micro nutrients such as phosphorus, potassium, zinc, magnesium, manganese. Although the mechanism of action of yeast on plant growth is not known yet, studies have shown that it may be a bio-agent. The indirect effect is related to the protection of plants from stress conditions. (Ibrahim et al., 2019) stated that yeast has the effect of suppressing pathogens and promoting the stress tolerance activity of the plant.

## 2. THE USE OF YEAST in PLANT CULTIVATION

Today, active dry yeast as a natural biostimulant has a positive effect on the growth and yield of many crops, while it has become a friendly product for humans, animals and the environment as an alternative to chemical fertilizers used recently. Unfavorable conditions for plants such as global climate change, natural erosion, desertification, and decrease in soil fertility, which have made their effects felt more recently, have revealed the necessity of increasing plant resistance (Tripathi et al., 2019). Phytohormones, amino acids and vitamins in yeast play a vital role in tolerance of environmental stress in plants. When the effects on plants are examined; It has been observed by Hammad (2008) that it stimulates cell division and expansion, chlorophyll formation, protein and nucleic acid synthesis and alleviates the harmful effects of abiotic stress factors such as drought stress.

When studies with different plant groups were examined, it was found that it reduced drought stress on pea plants (*Pisum sativum* L.) and increased their growth and yield (Xi et al., 2019). In a study on lupine, active dry yeast of the plant; vegetative growth has been shown to improve salinity stress tolerance by regulating chlorophyll, carotenoids and total soluble sugar content, enzyme activity and osmolytes (Taha et al., 2021). Yeast and amino acids were applied to the wheat plant under drought stress and it was seen that the applications reduced the negative effects of drought and active dry yeast was more effective than amino acid (Haider et al., 2021). Active dry yeast application in Caraway (*Carum carvi* L.,) plant increased vegetative growth, N, P, K, Ca, Mg,

Fe, Zn, Mn and total carbohydrates and fat ratio in plant roots and green parts (Medani & Taha., 2015). It has been shown that it increases the essential oil yield, plant nutrition and growth in thyme plant (El-Leityh et al., 2006). In the study conducted on Salvia officinalis plant, 5 g/L active dry yeast extract used from the leaves significantly increased the plant height, leaf area, fresh and dry weights of leaves and plants, and dry weight of stems (Al-madhagi, 2019). In the study of active dry yeast and humic acid in Cucumber plant, it was noted that yeast increased yield alone or with a combination of humic acid, and also showed a synergistic effect with humic acid (Rageh & Abou-Elwafa, 2017). Shehata et al., 2012 investigated the effect of active dry yeast using foliar (1, 2, 3 or 4 g L-1) ratios on cucumber yield and component and evaluated cucumber yield, fruit/plant number, fruit length, fruit diameter and average showed a significant increase in fruit weight. It was observed that all growth and yield parameters examined in the melon plant applied foliar with yeast produced higher values than the control group (Abdel Nabi et al., 2014). In another study, foliar application of active dry yeast extracts increased plant length, stem, leaf number/plant, leaf area/plant, fresh and dry weights, and tuber yield of potato plants (Ahmed et al., 2013). It has been observed that active dry yeast used alone and together with aloe vera extract to the basil plant increases the yield and quality of the plant (Hamouda et al., 2012). It was observed that active dry yeast increased plant height, number of branches, leaf, stem, root, flower, fresh and dry weight, as well as chemical components, percentage of essential oil content in Ruta graveolens plants (El-Sherbeny et al., 2007). El-Leity (2006) reported

that the use of active dry yeast significantly increased vegetative growth and oil production compared to Salvia officinalis control plants. It showed that spraying of active dry yeast used on Canino Apricot trees, alone or in combination with Boron and GA3, resulted in a significant increase in leaf mineral status, yield and fruit quality compared to control application (Hassan et al., 2005). It has been reported that the application of yeast extract increases the tolerance of the plant against heavy metal stress due to the hormones, sugars, amino and nucleic acids, vitamins and minerals it contains (Abdo et al., 2012). In general, when all studies were examined, it was found that active dry yeast had a beneficial effect on the growth, yield and chemical components of plants (Mohamed, 2005). In addition to increasing fruit yield and quality, it is also used as a biostimulant to improve the storability of products (Shaaban et al., 2015). The positive effect of active dry yeast on reducing fruit rot is that it can produce hydrolytic enzymes that can act on the cell walls of pathogens and extracellular polymers that appear to have antifungal activity, such as phytoalexins (acoparone and scopoletin) in citrus fruits (Sallam et al., 2012). When other studies were examined, it was used to prevent postharvest deterioration of tomato (Yan et al., 2008), to preserve fruit firmness of papaya (Guo et al., 2014) and to reduce weight loss of orange fruits during storage (Sallam et al., 2012).

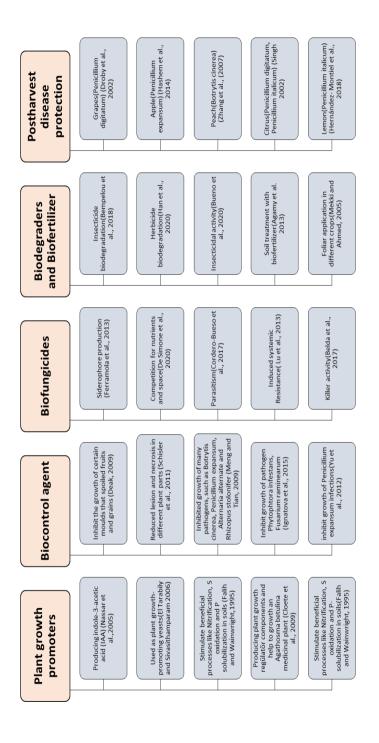


Figure 3. Applications of yeast in sustainable agriculture as plant growth promoters, biocontrol agent, biofungicides, biodegraders, biofertilizer and postharvest disease protection.

## 3. USE OF ACTIVE DRY YEAST in ANIMAL FEEDING

With the restriction of the use of growth promoting chemical products in animal feeds, the necessity of using alternative natural products has emerged. The use of yeast, one of these products, has increased significantly in recent years with feed ingredients produced from various fermentation processes (Shurson, 2017). Yeasts, which have been used in animal nutrition for many years (Stone, 2002), gain importance due to their efficacy of activating microorganisms that convert lactate to short-chain fatty acids, increasing digestibility, optimizing volatile fatty acid ratios, reducing ammonia-nitrogen and pH fluctuations, and increasing microbial activity in the rumen (Cagle et al., 2020). The official definitions and international feed names of major yeast products and feed containing yeasts approved for use in animal feeds are listed in the AAFCO (2017). Stone (2002), reported that the most common yeast used in the animal feed industry is active dry yeast with a dry matter content of 95%. In studies with active dry yeast, it was stated that by improving the ruminal environment, it increased milk production and feed efficiency and reduced liver abscess. (Crossland et al., 2019). It was determined that milk yield, weight gain, digestibility of food, cellulolytic bacteria counts increased in ruminant animals (Sartori et al., 2017). In addition, thanks to its probiotic feature, it has been found that it regulates the intestinal microbial balance and eliminates digestive disorders (McAllister et al., 2011). Active dry yeast promotes the proliferation of cellulolytic bacteria and ensures the breakdown of microbes, especially ADF and

NDF. It has been observed that yeast, which is used as an additive to silage corn, facilitates the digestion of corn and accelerates the breakdown of NDF (Guedes et al., 2008). In a study investigating the growth performance, carcass characteristics, meat quality and blood index values of bulls, two different yeast products were used. When the effect of active dry yeast and yeast cultures used at the end of the 98day trial was examined, it was concluded that active dry yeast was more effective in improving the growth performance and carcass characteristics of bulls. In addition, it was observed that both active dry yeast and yeast cultures positively changed meat quality and blood indices related to fat metabolism (Geng et al., 2016). In another study investigating the effects on growth performance, rumen microbial composition and carcass performance of beef cattle, it was determined that active dry yeast increased the average values compared to the control group (Liu et al., 2021). Active dry yeast supported the digestibility of nutrients in the small intestine of beef cattle in dietary supplementation studies (McAlliste et al., 2011), carbohydratedigesting bacteria (Ruminococcus albus, R. champanellensis, R. bromii and R. obeum) and lactate-using bacteria (M. elsdenii, Desulfovibrio desulfuricans and Desulfovibrio vulgaris) population (Ogunade et al., 2019). In a study conducted in sheep, it was determined that there was an increase in the cis-9, trans-11 CLA and vitamin A content of sheep meat after yeast supplementation (Milewski et al., 2012). Similarly, it was observed that with yeast added to the feed ration of lambs, fatty acids such as cis-9, trans-11-CLA, C14:1, C18:2 and C22:6 in intramuscular fat reached high levels (Milewski & Zaleska., 2011). The use of active dry yeast as a feed additive in kids has been found to improve properties such as live weight gain, nutrient digestibility and rumen fermentation (Pradhan, 2021).

## **4.CONCLUSION**

Environmental problems caused by the wrong and unconscious use of chemical-containing plant nutrition and plant protection products used to increase productivity in agricultural production are accepted as undisputed. With the rapid population growth, the need for agricultural products will increase and accordingly the use of traditional fertilizers will increase. This increase will cause the accumulation of pollution to accelerate. Therefore, new agricultural strategies should be developed. The inclusion of organic materials in agricultural production for sustainable agriculture and the environment is an important step in solving problems. It has been embodied by the studies that the minerals, amino acids and vitamins in the active dry yeast promote plant growth and development. Dry yeast has recently attracted attention as an alternative growth agent in the bio-organic fertilization system. At the same time, it is noteworthy as a bio-agent worth researching and developing with its indirect effects such as increasing the resistance of the plant to stress conditions and suppressing pathogens. The use of yeast in agriculture is an interesting topic with potential. The discovery of a yeast that can be used in many areas of the agricultural sector can be an important study. With innovative studies on this subject, it seems possible to develop an agricultural yeast that increases plant

productivity and supports sustainability with its environmentally friendly feature.

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#### **CHAPTER 4**

## MICROBIAL FERTILIZERS AND ITS EFFECT MECHANISM IN PLANT PRODUCTION

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#### **INTRODUCTION**

Microbial fertilizer (biological fertilizer) is a formulation of living, pure or mixed microorganisms that colonize in the rhizosphere or enter plant tissues when applied to seeds, plant surface or soil, fix atmospheric nitrogen, increase the uptake of soil phosphorus and other plant nutrients and plant growth (Cakmakçı, 2014). Within the scope of the "Regulation on the Production, Import, Export and Market Placement of Organic, Organomineral Fertilizers and Soil Conditioners Used in Agriculture and Other Products with Microbial, Enzyme Content and Organic Origin" of the Ministry of Food, Agriculture and Livestock, published in the Official Gazette dated March 29, 2014 and numbered definition is "commercial 28956. Its formulations of live microorganisms that play a role in providing the nutrients required for the plant" (Anonymous, 2014). Microbial fertilizers; (biological fertilizers, bio-fertilizer, bacterial fertilizers, bio-inoculants, bacterial inoculants, microbial cultures) refers to the commercial formulations of live microorganisms that are involved in providing the nutrients required for plants and making them useful in a biological way. Biofertilizers are also known as microbes applied to the soil either directly or indirectly (Owen et al., 2015). Conformity assessment of the fertilizers currently being used within the scope of the "Regulation on the Production, Import, Export and Market Placement of Organic, Organomineral Fertilizers and Soil Conditioners Used in Agriculture and Other Products with Microbial, Enzyme Content and Organic

Origin" published in the Official Gazette dated 23 February 2018 and numbered 30341. It is done by the Ministry of Forestry.

The most important group used as microbial fertilizer is bacteria. The abbreviation of "Plant Growth Promoting Rhizobacteria" is PGPR and it can be defined as "Plant Growth Promoting Bacteria" in Turkish. This definition was first used by Kloepper et al., (1980). These bacteria are mostly Acetobacter, Acinetobacter, Achromobacter, Aereobacter, Agrobacterium, Alcaligenes, Artrobacter, Azoarcus, Azospirillum, Azotobacter, Bacillus, Beijerinckia, Burkholderia, Chromatium, Clostridium, Enterobacter, Envinia, Flavobacterium, Herbaspirillum, Klavobacter, Kleink, Microbacterium, Microcillus. pseudomonas Rhizobium belongs to the genera Rhodobacter, Rhodosprilum, Serratia and Xanthomonas. According to the studies carried out to date, it is clearly seen that species belonging to Azotobacter, Acetobacter, Azospirillum, Burkholderia, Pseudomonas, Bacillus and Paenibacillus genera stand out as PGPR (Çakmakçı et al. 2014). In order for a bacterium to be a bacterium that promotes plant growth; It must be colonized in the root zone, it must be able to spread easily in the root rhizosphere, it must activate plant growth and protection (Ahemad and Khan, 2011)

Investments on microbial fertilizers have been increasing in the world since 1980. Fertilizers named as "Bio-fertilizers, Microbial Fertilizers or Microbial Inoculation Material" at the National Specialized Conference held in Beijing on 30-31 October 1995 increased the crop yield, improved soil fertility and bioavailability, reduced the need for

chemical fertilizers, decomposed organic wastes and fed nutrients. It has been reported that it releases its elements and as a result reduces environmental pollution, its use in ecological agriculture is economical compared to other fertilizers and it is ideal fertilizer for foods that are eaten green. As a result of the decisions taken at this conference, it is seen that the use of these fertilizers has become widespread today (Chen and Xoing, 1997). Indeed, Kour et al. (2019) stated that the technology used in the production of biofertilizers is quite simple and the installation cost is much lower.

There are many commercial formulations registered as microbial in the world and their numbers are increasing rapidly. Bacillus subtilis; barley, cotton, legumes, rice, tomato, pepper, broccoli, ornamental plants, melon, lettuce, Bacillus amyloliquefaciens; tomatoes, peppers, broccoli, ornamentals, melons, lettuce, Pseudomonas fluorescens; Pseudomonas syringae in apple, cherry, cultivar, peach, almond, pear, strawberry, potato, tomato; citrus, pome fruit is commercially licensed (Chet and Chernin, 2002).

The most distinctive features of microbial fertilizers regarding plant growth are symbiotic and non-symbiotic nitrogen fixation, making plant nutrients available, biological control of soil-borne diseases and secretion of plant growth stimulating substances. Bacteria that live freely in the soil, encourage plant growth, help plants control pathogenic microorganisms, and can also be used as biological fertilizers are called "Plant Growth Promoting Rhizobacteria" or PGPR (Plant Growth Promoting Rhizobacteria). In order for these bacteria to

receive this name, they must have three essential features (Ahemad and Khan, 2011).

#### These features are:

- To be colonized in the root zone.
- To be able to spread effectively in other microhabitat in contact with the root, to be effective in increasing plant growth and protection,
- Ability to promote plant growth

Microorganisms such as fungi and algae, especially bacteria, are used as microbial fertilizers in agricultural production. However, it is necessary to mention two major difficulties encountered in the use of microbial fertilizers. The first of these is that if the fertilizers cannot be stored under appropriate conditions, they lose their vitality and the fertilizer cannot fulfill its function. The second is that if the soil conditions are not suitable for the applied organisms, the effect of the fertilizer cannot reach the desired level. For this reason, special storage conditions of microbial fertilizers should be taken into account and the properties of the applied soils such as moisture, organic matter, pH, which affect the life of microorganisms, should be controlled. Thus, the effect period of the application made by creating a natural ecosystem is extended and the activities of microorganisms can develop spontaneously (Karacal and Tükenkçi, 2010).

#### Classification of microbial fertilizers

Microbial fertilizers are classified according to their microorganism content and mechanism of action as follows (Li, 2001).

According to the microorganism contents:

- Fertilizers Containing Bacteria (Fertilizers containing bacteria that form nodules, fix nitrogen and dissolve phosphorus)
- Actinomycete Containing Fertilizers (Antibiotic containing fertilizers)
- Fungicide Containing Fertilizers (Mycorrizal fungi-containing fertilizers)

Microbial fertilizers according to their mechanism of action:

It is possible to divide the mechanisms of action of microbial fertilizers into two as direct and indirect (Aşkın et al., 2014).

#### **Direct effect mechanism**

- Biological nitrogen fixation
- -Organic and inorganic phosphate solubility
- -Plant hormone production (auxin, cytokinin, giberallin, aminocyclopropane carboxylate deaminase)
- Increasing nutrient intake
- Siderophore production
- Increasing antioxidant enzymes and resistance to stress

- Vitamin production (niacin, pantothenic acid, thiamine, riboflavin, biotin)
- Soil aggregation

#### **Indirect mechanism of action**

- Antibiotic production
- Do not colonize roots
- HCN production
- Production of antifungal metabolites
- -Prevention of pathogens through compet

#### Commonly used types of microbial fertilizers:

A-Fertilizers containing nodule bacteria: Fertilizers containing nodule bacteria have been used for a long time in our country as well as in the world. In our country, the Central Research Institute of Soil, Fertilizer and Water Resources (TGSKMAE) has a special Rhizobium collection for each legume (soybean, pea, bean, broad bean, vetch, clover, clover) and is produced according to demand and delivered to producers (Anonymous, 2014). Within the nodule-forming fertilizer are Rhizobium, Bradyrhizobium, Sinorhizobium, Azorhizobium, Mesorhizobium and Allorhizobium species that fix the atmospheric nitrogen form suitable for the plant and provide nodule formation in legume roots (Vance, 1998; Graham and Vance, 2000).

**B-Fertilizers containing nitrogen-fixing bacteria:** Free-living bacteria in the soil, such as Azotobacter, Beijerinckia and Clostridium, are capable of fixing atmospheric nitrogen without the need for any plant roots (Benson and Silvester, 1993). There are also various Azotobacter species naturally found in the soil and there is no seasonal limitation for fixation. The amount of nitrogen bound by Azotobacters is generally lower than Rhizobium species (Li and Zhang, 2001).

#### C-Fertilizers containing bacteria that break down silicate minerals:

This fertilizer group, also called "Bio-Potassium Fertilizers", generally includes bacterial species such as Bacillus mucilaginosus, Bacillus circulans and Bacillus macerans. These species decompose the soluble potassium minerals in the solvent medium and if they are applied to the soils with potassium deficiency, they have a positive effect on the yield (Xiong et al.,1993; Peng and Ye 1995). These bacteria provide a competitive environment by inhibiting the activities of other microorganisms that have a negative effect on plant development in the rhizosphere with the microbial metabolites they produce, and facilitate the uptake of plant nutrients by promoting plant growth (Li, 2009).

**D-Fertilizers containing antibiotic-producing bacteria:** In these antibiotic-producing fertilizer groups, strains of the genus Actinomyces microflavus (for example, strain 5406) are generally included. These species are widely used especially in China. These fertilizer types are effective on many diseases as well as their positive effects on soil fertility and plant growth (Wu and Li, 1994). In addition, among some

Bacillus and Pseudomonas species, some are defined as PGPR bacteria (Schippers et al., 1987).

E-Fertilizers containing more than one microorganism: In recent years, microbial fertilizers containing more than one microorganism registered in our country have been developed. The most important feature of these derivatives is that they should not have an antagonistic effect between microorganisms.

They can be grouped into three groups according to their combination (Bilen, 2014):

- 1-Inoculation materials containing mixed strains: For example, combining 3 different strains of Bacillus cereus (Xu et al., 1991).
- 2-Inoculation materials containing different bacteria: For example, combining Azotobacter and Bacillus spp. (Higa, 1996).
- 3-Bacterial derivatives containing chemical or organic fertilizers: (Bioactive Combined Fertilizer) Contains rare elements, macro and micro elements and organic wastes in the earth's crust (Deng et al., 1993; Yu et al., 1998; Chen et al., 1998).
- F-Fertilizers containing phosphate bacteria: The conversion of organic phosphorus to inorganic form in the soil and the dissolution of inorganic phosphate occur with metabolites secreted by soil-borne bacteria, fungi and actinomycetes. As phosphate bacteria, Bacillus spp., Thiobacillus thiooxidans species and Pseudomonas and Atrhrobacter genera are capable of degrading phosphorus (Ge and Wu, 1994).

Phosphorus is one of the basic elements limiting plant growth in soil and is available in organic and inorganic forms. Phosphorus in organic form constitutes approximately 20-80% of the total phosphorus. While the organic form consists of humus, vegetative, animal and microbial tissues, the remaining inorganic form passes into the soil solution with chemical fertilizers and weathering and erosion of the bedrock (Ahemad and Khan, 2011). Since phosphorus is generally insoluble in soils, available P for plant growth is insufficient. Although phosphorus fertilization is done regularly, the uptake efficiency of the plants is low. Production of acid or alkaline phosphatase and siderephore by plant roots or microorganisms, release of organic anions, hydrolysis of soil phosphorus, release of soil organic phosphorus or separation of P from organic wastes make phosphorus available to plants. Among the bacterial communities in the rhizosphere, Pseudomonas and Bacillus species stand out as effective phosphate solvents. Along with Penicillium and Aspergillus fungi, bacterial species belonging to Pseudomonas, Bacillus, Rhizobium and Enterobacter genera, and especially Bacillus megaterium, Bacillus circulans, Bacillus subtilis, Paenibacillus polymyxa, Bacillus sircalmous and Pseudomonas striata species are the most common phosphate solvents.

While agricultural lands have high values in terms of phosphorus reserves, the amount available to plants is paradoxically a small part of all this reserve. Plants that cannot benefit from organic phosphorus in the soil can only take phosphorus in the form of monobasic (H2PO4 - )

and dibasic (HPO4 -2) phosphate ions, which are two useful forms (Okur, 2014)

Phosphorus solvent bacteria (PSB) promote plant growth by increasing inorganic and organic P solubility and uptake through different mechanisms such as proton removal, in which organic acid secretions and various metabolites, especially phosphate solvent gluconic acid, take part (Khan et al., 2006; Çakmakçı, 2014)

The factors determining the effectiveness of biological fertilizers containing phosphate-dissolving bacteria are given below (Çakmakçı and Erdoğan, 2005):

- 1. Quality of inoculum
- 2. Plant variety
- 3. Culture conditions
- 4. Soil properties
- 5. Temperature and humidity regime
- 6. Soil structure
- 7. Vaccination and application technique
- 8. Availability of usable substances
- 9. Fertilization level It has been proven by many studies that plant yield increases by inoculation of phosphorus-dissolving bacteria into the soil and plants.

#### **Biofertilizer Bioformulations**

Today, there are a wide variety of commercial biofertilizer formulations produced from different microorganisms. Under normal conditions, the shelf life of biofertilizer preparations is very short (Mahdi et al., 2010). Many different strategies are used to further extend this shelf life. Different strategies can be used to achieve this long shelf life, such as optimizing the biofertilizer formulation, using thermotolerant/drought resistant/genetically modified strains, or making liquid formulations (Brar et al., 2012). The formulations may be prepared in solid or liquid forms.

In addition to the carrier material, the formulations also contain auxiliary nutrients and/or protectors such as trehalose, molasses, mactose, sucrose and glycerol. In this way, the shelf life of the commercial product is further increased (Brar et al., 2012).

Another formulation strategy is liquid formulations. These formulations are promising formulations. Because it contains enough nutrients and cell protectors for the cells to maintain their vitality. In fact, studies have shown that solid formulations for some organisms retain their activity for six months, while liquid formulations maintain their activity for up to two years. In addition, the cost of liquid formulations is higher than solid formulations and the risk of contamination is high (Brar et al., 2012; Mahdi et al., 2010).

### The benefits of microbial fertilizers and their application in crop production

Today, the recognition of microorganisms used in agriculture as fertilizer and inoculation material is increasing and their use is becoming widespread. Microbial fertilizers, which are composed of microorganisms living in symbiosis with the plant or living freely in the soil, gain importance in obtaining optimum products by ensuring the effective use of organic and mineral fertilizers used in organic and conventional agriculture by the plant.

The benefits of these fertilizers can be summarized as follows:

- It meets the amount of nitrogen needed by plants by binding the nitrogen in the atmosphere to the soil (in the form of organic nitrogen).
- It ensures that the phosphorus accumulated in the soil and is unusable is taken up by the plants.
- Accumulation of inorganic substances and minerals in the soil resulting from the application of chemical fertilizers is prevented, it is environmentally friendly.
- It regulates the pH level in the soil; It brings the phosphorus, potassium and nitrogen in the soil to the ideal level.
- The same degree of efficiency and success is achieved in suitable soil conditions and climate.

It helps to prevent desertification and erosion by increasing the amount of organic matter in the soil.

- Provides plant development.
- Provides resistance stimulating agents against pathogens.
- It protects the plant against pathogenic microorganisms.
- It produces the growth enzymes (auxin, indolacetic acid, gibberellic acid, etc.) it needs and regulates absorption. In addition, there is no need to add artificial enzymes again.
- Thanks to the enzymes in the biofertilizer content, it ensures the decay of the roots and organic materials left in the soil after harvest and brings the harvest stubbles to the soil as humus (Katırcıoğlu et al., 2014).

While there are many studies on the effects of microbial fertilizers on the useful nutrient content of soils, plant growth and nutrition, studies on the effects of these preparations on biological parameters, which are important indicators of soil fertility, such as soil enzyme activity and the presence of microorganisms, are very limited.

# Some of the studies conducted in the last ten years using microbial fertilizers;

In a study conducted by Çubuklu (2011) in open field in Çanakkale region, the effects of PGPR usage on yield and quality in grafted and ungrafted tomato cultivation were investigated. Trichoderma harzianum bacteria are used as microbial fertilizer; Kemerit rootstock

Veglia RZ F1 tomato variety was used as plant material. As a result of the research, the best results were achieved with grafting and microbial fertilizer application, and the yield per plant was found to be approximately 2.5 times higher than the control group. The lowest results were obtained from control plants. Yield and quality parameters were found to be higher than the control group, as grafting and microbial fertilizer applications increased root growth, provided phytohormone production, and facilitated nutrient uptake from the soil. The researcher also stated in his study that grafting and microbial fertilizer applications increased vitamin C in fruits.

The highest yield per plant (17,258 kg/plant in the 1st planting, 9,1891 kg/plant in the 2nd planting) was obtained with the microbial fertilizer T. harzianum and grafting; The lowest yield (7,705 kg/plant in the 1st planting, 3,445 kg/plant in the 2nd planting) was obtained from the control plants without both applications. It was stated that the height, root fresh weight and yield values of the applied plants were higher as a result of both factors promoting root development, producing hormone-like metabolites, and better dissolving nutrients from soil or organic matter. However, it was determined that the interaction of both factors significantly increased the vitamin C content in fruits. In addition, as a result of the observations, it has been said that the grafted plants are more resistant to soil-borne diseases and pests, as well as to stress conditions such as low temperatures.(Çubuklu, 2011)

Jaipaul et al. (2011) conducted a two-year study on pepper and peas in India, organic (farm manure, chicken manure and vermicompost),

microbial (azotobacter, Rhizobium, phosphorus-dissolving bacteria), chemical (in peas: 2.5 kg N + 7,  $5 \text{ kg P}_2\text{O}_5 + 5 \text{ kg K}_2\text{O} + \text{farm manure}$ 500 kg/da) combined with fertilizers have investigated the effects on yield and yield characteristics. In terms of the properties examined in the study, the highest plant height in peas was 93.6 cm, while it was obtained with chicken manure (500 kg/da) + microbial fertilizers, and this value was followed by the plots where the combination of chemical + farm (500 kg/da) + microbial fertilizer was given. The lowest plant height value (72 cm); only in the parcels where organic fertilizers were combined [farm manure (1 ton/da) + chicken manure (150 kg/da) + vermicompost (150 kg/da) + microbial fertilizer] were found. Application where chemical and organic fertilizers are combined; It showed the same positive effect on the pod length (10.1 cm) and the number of seeds per pod (9.1 units), and it took the first place in pod yield per decare. When the results of the study are examined in general, it is observed that organic fertilizers increase the development and yield of peas; It is understood that the highest effect is achieved in the application where chemical and organic fertilizers are combined, while the lowest values are achieved with the combination of farm manure (2) tons/da) + microbial fertilizer.

ISR 2000, Crop-Set, Manda 31, Vitormone, Biosaps and Fosfert as plant activator and microbial fertilizer in the study conducted to investigate the effect of microbial fertilizers and plant activators used in organic cultivation in Konya province Karapınar between 2010-2011, on the yield and quality characteristics of Maestro carrot variety.

used. As a result of the study, the effect of plant activators and microbial fertilizers on yield was found to be statistically insignificant; The highest yield was obtained from the Crop-Set (7.171 kg/da) application, followed by the ISR 2000 (6.977 kg/da) 34 application and the conventional (6.825 kg/da) application, respectively; The lowest yield was reported to be obtained from the Buffalo 31 (6.648 kg/da) application. (Kiraci et al., 2012).

Dasgan et al., (2012) used complete nutrient solution (100%), nutrient solution in which all elements were reduced by 20% and all elements were reduced by 40% in their study, in which they examined the effect of using some microorganisms as biofertilizers in soilless pumpkin production on reducing the amount of nutrients used. As biofertilizer, (a) mixture containing 9 types of mycorrhiza (Glomus intraradices, G. mosseae, G. aggregatum, G. clarum, G. monosporus, G. deserticola, G. brasilianum, G. etunicatum, Gigaspora margarita), (b) Basillus subtilis, (c) Azoto bacter vinelandi, Clostridium pasteurianum, and (d) microalgae Chlorella spp. used. It was determined that mycorrhiza and micro-algae application increased the yield values in applications where the nutrient material was reduced by 20% and 40% compared to the control. Mycorrhiza application provided the preservation of plant growth, which was destroyed by the reduction of fertilization, and it was observed that fruit weight and quality values did not change. Researchers have stated that up to 40% of nutrients can be reduced in soilless agriculture with the application of mycorrhiza.

Göksu (2012), in 2008 and 2010, in Bursa-Görükle and Bursa-Yenişehir locations, organic (chicken manure), microbial fertilizer (nitrogen-fixing Bacillus megaterium BA142 and phosphate solvent Bacillus megaterium M3) and chemical fertilizer (46%) in two different pea varieties. N, 46-48% P<sub>2</sub>O<sub>5</sub>) applications have examined the effects of yield and yield characteristics and protein ratio. In the research; They determined that the lowest values in terms of plant height, number of pods per plant, pod length, number of seeds per pod, number of seeds per plant, 1000 grain weight, grain yield and protein ratio were obtained from the control plots, while the highest values were obtained from 1 NP application. They reported that microbial fertilizer (BA142 and M3) applications made a positive contribution in terms of the properties examined, but phosphorus and nitrogen biofertilizer application in pea production could not be an alternative to commercial phosphorus and nitrogen fertilizer application.

If the results of the three trials will be summarized in general; the microbial fertilizers, plant activator and extract used are not as effective as nitrogen fertilizers; Phosphorus fertilizers, like biological fertilizers, cannot have the desired effect on the development and quality of grass plants; There is no significant difference in turf performance between English grass and Reed ball, but Reed ball has a slightly higher hay yield in general; It has been concluded that for the desired growth, yield, quality and turf performance in turf plants, depending on many factors such as plant type, soil structure, climate and growing conditions, 5.0-7.5 kg nitrogen per decare would be ideal. In addition, it has been

concluded that it is difficult to predict the possibility of an epidemic of buckthorn disease and it is difficult to fight the disease, the chemical and biological fungicides used in the fight against buckthorn prevent the infection at low rates, but do not show sufficient effect, and the preparations used give unstable results in terms of control of the disease (Surer, 2013).

In a study (Hridya and Byju, 2014), eight different types of microbial fertilizers (Azospirillum, Mycorrhiza and phosphorus solvent bacteria), NPK fertilizers and biocontrol agents (Trichoderma and Pseudomonas fluorescens) were applied in various combinations in a study on cassava plant grown in tropical climates and used as a raw material in the industry, and the effects on the chemical, biochemical and some biological properties of soils were investigated. At the end of the study, it was determined that urease activity was higher in the area where Azospirillum + mycorrhiza was applied, compared to other applications. It has been reported that 1/2 NPK+Trichoderma+Mycorrhiza application significantly increases and β-glucosidase dehydrogenase activity compared other applications.

Kotan (2014), in the study in which the effects of microbial fertilizer applications on various plants, as well as the yield, were examined at some molecular level; BmCoton Plus application, which consists of bacteria producing free nitrogen fixers, hormones, amino acids and organic acids, provides an increase in yield of up to 100% in cotton and silage maize. They reported that the period was shortened and the yield was increased by increasing the number of forms. In addition, they stated that due to the production of chitinase (the enzyme used in plant nutrition as a biological control agent against insects by breaking down the chitin structure of crustaceans) in the content of the product, some disease severity and pest populations were reduced.

Tunc, (2015) Microalgae, which is completely natural and easy to produce, was tested on wheat, which was selected as a pilot plant, and its biofertilizer potential was examined. Within the scope of the study, incubation, greenhouse and field trials were carried out, and as a result of the study, pH, EC, nitrate nitrogen, ammonium nitrogen, total nitrogen, organic carbon, C/N ratio, cation exchange capacity, aggregate stability, plant-available phosphorus, available potassium, Ca, Mg, Mn, Fe, Zn, Cu, Co, Cd, Ni, Pb and urease, beta glucosidase and alkaline phosphatase enzyme activity determinations, plant height, yield, protein, N, P, Fe, Cu, Zn, Mn, Ni, Cd, Pb, Co analyzes were made. The results showed that Oscillatoria amoena (OA) was the most effective species among the 10 algae species tested, and more detailed dose studies (alone and in combination with chemical fertilizers) for the use of this microalgae species as a microbial fertilizer and field crops in wet and dry conditions with different plant varieties. It was stated that it would be useful to establish experiments. Currently, there are microalgae fertilizer products on the market that are produced abroad in our country. Only these products are imported, there is no commercial domestic production. In this study, the usability of microalgae as biofertilizer was investigated, and wheat was preferred

as a pilot plant. The reason why wheat was chosen as a pilot plant; It is the most produced agricultural product in Turkey and in the world. In addition, 20% of the calories provided from food in the world belongs to wheat. Due to the elasticity of gluten, it is an unrivaled plant suitable for bread making. The fact that its agriculture is easy and completely machine-based leads the growers to wheat farming. The fact that it has a very high compensatory ability, can compensate for the mistakes of the grower and adverse conditions to a certain extent, gives wheat a different place among the cultivated plants. Due to these characteristics, wheat has stated that it will continue to be a strategic plant in the future, as it has been in the past and today (Anonymous, 2021).

Plants are under stress due to the salinity problem in agricultural lands. Under stress conditions, some physiological properties such as osmotic pressure, cell membrane permeability, photosynthesis are adversely affected in plants and as a result productivity decreases. In this context, the effect of brassinosteroid (BS) and biological fertilizer applied to corn plant grown under saline conditions created by applying (100 mM) NaCl was investigated. As a result of the study, salt stress caused a decrease in the total chlorophyll, leaf water potential, Ca, K, N, P uptake and photosynthesis amount of plants, while it increased osmotic pressure, cell membrane permeability and Na uptake. With the application of BS and biological fertilizers to the corn plant, the negative effects of salinity in the plants were partially improved. It has been reported that foliar EBR (24-Epibrassinolide) applications, especially at doses of 1.5 and 2 µM, partially increase salt tolerance in

corn plants grown in salty conditions. Effective doses of these compounds can be recommended for maize cultivation in saline conditions. The positive effect of EBR applications on corn plant grown under salt stress; It is due to reducing the accumulation of Na in the plant and increasing the amount of N and Ca in the plant. In addition, it was stated that this positive effect was also due to the improvement in other tested physiological parameters (such as cell membrane permeability) (Altaş, 2016).

Cevheri, (2016) in the experiment, organic farm manure (200 kg/da), pigeon manure (100 kg/da) and microbial fertilizer (100 cc/100 lt water) were applied. It was determined that the experiment was carried out in four replications according to the split plots trial design. According to the combined analysis results of two years; the seed cotton yield of the cultivars varied between 387.66 kg/da (ST 468) and 399.80 kg/da (BA 119) and the highest seed cotton yield was obtained from BA 119 variety. As a result of organic and microbial fertilizer applications, it was determined that the seed cotton yield ranged between 275.70 kg/da (control) and 437.82 kg/da (pigeon manure + microbial fertilizer) and the highest yield was obtained from pigeon manure + microbial fertilizer application. It has been determined that the microbial fertilizer application has a significant effect on the seed cotton yield. It has been determined that organic and microbial fertilizer applications provide a statistical increase in plant height, wood branch number, fruit branch number, first fruit branch node number, number of bolls per plant, boll

weight, boll seed weight, 100 seed weight, gin yield and fiber index values.

Özaktan, (2017) in his experiment, 2 kg of humic acid (2 applications per decare) was applied to the main plots, microbial fertilizer with Bacillus pumilus C26 phosphate dissolving feature (2 applications) to the sub-plots, and 0, 7.5, 15 and 22.5 hectares to the sub-gold plots. kg/da (4 applications) phosphate rock (29.3% P<sub>2</sub>O<sub>5</sub>) was applied (2x2x4x4=64). At the end of the trial period, plants were sampled from each plot. In his samplings, he examined yield and yield elements in beans. As a result of the research, it was stated that the doses of humic acid, microbial fertilizer and phosphate rock had different effects on the bean plant in terms of the investigated properties. In the two-year average values; flowering days 47.38-49.00 days, physiological death days 91.75- 95.38 days, plant height 42.95-53.65 cm, first pod height 10.16-13.90 cm, number of major branches per plant 2.36-2.84, number of pods per plant 13.85-24.85, seeds per plant The number of seeds per pod is 38.64-59.83, the number of seeds per pod is 2.36-3.16, the grain yield per unit area is 173.8-314.3 kg/ha, the harvest index is 31.25-39.73%, the biological yield is 274.4-382.0 kg/ha, the hundred-seed weight is 38.92-43.10 g, and the grain yield is 38.92-43.10 g. The protein ratio was found to be 22.93%-24.94%. In particular, the highest dose of phosphate rock (22.5 kg/da phosphate rock) along with humic acid and microbial fertilizer applications achieved the highest grain yield with 314.3 kg/da on average over the years. Compared to the control plots (173.8 kg/da), the effect of yield increase was found to be

significant in all applications. It has been stated that for sustainable agriculture in conventional and organic bean cultivation, application of humic acid and microbial fertilizers together with phosphate rock at 22.5 kg/da (29.3% P2O5) is most suitable for high grain yield.

Germination and emergence rate, plant height, leaf area, root length, root fresh weight, root dry weight, stem fresh weight, stem dry weight, chlorophyll content of T. harzianum applications (10, 15 and 20 gL-1) compared to control. 10 gL-1 excluded) and showed increased yield. In general, they observed that the 15 g.L-1 dose was prominent. (Özbay et al., 2018).

Kour et al. (2019) discussed the diversity of microorganisms that promote plant growth, their commercial aspects, and technologies for the production of biofertilizers for agricultural and environmental sustainability. In their study, they aimed to reduce the amount of chemical fertilizers used in greenhouse tomato cultivation (Azeri F1) by using microbial fertilizers (Bacillus amyloliquefaciens FZB24(R) and Glomus spp.) and to determine the effectiveness of microbial fertilizer applications on plant growth, yield and fruit quality in reduced fertilization. They carried out in the Spring 2018 terms. Trial subjects; (1) 100% commercial fertilization (G); (2) 50% reduced commercial fertilization + Bacillus amyloliquefaciens bacteria (G/2 + FZB24(R)) and (4) 50% reduced commercial fertilization + Glomus intradices Arbiscular Mycorrhizal Fungus (G/2 + AMF). Before and after planting, physical and chemical analyzes were made in the soil and leaves, and the conditions of macro

(N, P, K, Mg, Ca) and micro (Zn, Fe, Mn, Cu) elements and plant growth (plant height, stem diameter, stem and root) Fresh and dry weight), yield (total and marketable yield) and fruit quality (fruit firmness, total water-soluble solids in fruit juice, pH, EC and Vitamin C) were compared. When the results of the soil analyzes are examined, it is observed that the EC values, which were low in the 2017 Autumn period, increased in the 2018 Spring period. Mycorrhiza fungus kept the EC value at a lower level. Our study stated that commercial fertilization increases soil salinity and EC value in tomato cultivation in Muğla province, where intensive greenhouse activities are carried out. Microbial fertilization reduced the negative effects on plant growth, yield and fruit quality under 50% reduced commercial fertilization conditions. Compared to full fertilization, the use of AMF resulted in a yield loss of only 1.11% in marketable yield in Autumn 2017. In the spring of 2018, this loss was observed as 3.81%. They observed that the use of microbial fertilizers is a good alternative to excessive fertilization, and especially the application of Glomus intraradices is more effective (Korkmaz, 2019).

In a study examining the effects of microbial fertilizer application in 4 different doses (0, 2, 4, 8 ml kg-1 seed) on plant growth and yield in sweet corn cultivation, the longest plant height and stem diameter were 174 cm in 8 ml kg-1 seed microbial fertilizer application. and 3.46 mm. The highest yield value was found to increase by 31.51% in 8 ml/kg seed application compared to the control. When the researchers compared their effects on yield and plant growth in general, they stated

that there was no significant difference between the doses of 4 and 8 kg-1, and it would be more beneficial to use a dose of 4 kg-1 from an economic point of view (Altunlu et al., 2019).

Candle (2020), A microbial fertilizer, eggplant (Solanum melongena L.) and cabbage (Brassica oleraceae var. capitata) seeds emergence rate, emergence time, seedling height, seedling thickness, epicotyl length, hypocotyl length, chlorophyll amount and plant nutrient element. Partner F1 eggplant and Fieldglory F1 cabbage cultivars were used as plant material in the research he carried out to determine the effects on their contents, and the seedlings were grown in a mixture of 70% peat + 30% perlite. Within the scope of the study, GS (traditional seedling cultivation), M-1 (irrigation with microbial fertilizer once after sowing), M-2 (irrigation with microbial fertilizers 2 times after sowing), M-3 (irrigation with microbial fertilizers 3 times after sowing), MB (soaking the seeds in water with microbial fertilizers), SB (soaking the seeds in water for 24 hours) were included in a total of 6 applications. According to the results of the research, it was observed that soaking eggplant and cabbage seeds in water with microbial fertilizers inhibited germination. The effects of microbial fertilizer applications on the percentage of emergence, average emergence time and germination index in eggplant were lower than the control group, and they shortened the emergence rate positively. In eggplant, seedling, hypocotyl and epicotyl length were highest in M-1, while true leaf number was highest in M-3, fresh weight M-1 and dry weight SB. Among the macro elements, N and P were determined the most in GS,

the content of K in M-3, Ca and Mg in M-1, while Mn from micro elements had the highest in M-2, Zn in M-1, Cu. was found in M-3. In terms of the criteria examined in cabbage, the highest emergence percentage was found in M-2, the shortest average emergence time in M-1, the highest emergence index in M-1 and the fastest emergence speed in M-3. While the highest seedling thickness value was observed in M-1, seedling length, hypocotyl and epicotyl length were observed in SB, chlorophyll amount was measured in M-2, fresh weight and dry weight were highest in M-1. While N, P, K from macro elements are found in M-2, the highest Fe content from micro elements is in M-1, Mn content is in SB, Zn content is in M-3 and Cu content is in M-1. has done.

Altunlu (2021), in the study, which aimed to investigate the effects of vermicompost and microbial fertilizer applications in the soil on plant growth, yield and quality in head salad (Lactuca sativa L. KG) and 4 different doses of vermicompost (0, 100, 200 and 400 kg da-1) and 2 different doses of commercial microbial fertilizer (M- and M+) were applied according to a randomized block design with 3 replications. Microbial fertilizer application was made twice as seed application and after planting. It has been determined that the application of 400 kg da-1 vermicompost and microbial fertilizer creates plant growth in the same way as chemical fertilization, the color and other quality criteria of the obtained plants are better maintained, and the nitrate accumulation, which poses a risk to human health, is significantly reduced compared to chemical fertilization.

Kotan et al. (2021), Bacillus subtilis TV-17C bacterial isolate, which fixes nitrogen, dissolves phosphate, dissolves calcium, produces amino acids and organic acids, and produces hormones, was formulated with an organic liquid carrier and used in apple sapling cultivation. The effects on the hormone levels in the plant, the levels of macro and micro nutrients in the plant and in the soil, and some soil properties were investigated. As a result of the research; found that this bacteria-containing microbial fertilizer formulation caused an increase in the hormone levels in the plant and in the macro and micronutrient levels in the plant and in the soil in all plant growth parameters in apple saplings.

Sen (2021) used nitrogen ( $N_2$ ) fixative and phosphate ( $PO_4$ ) solubilizing microorganisms for the biofilm seed coating to see the nutritional efficiency of the soybean seed period. analyzed with According to the results obtained, the effect of seed coating application on yield increase was found to be statistically significant. In addition, statistically significant effects on yield increase were found between cultivar and bacterial application interaction.

#### CONCLUSION AND RECOMMENDATIONS

Biological methods; It can be of microbial, vegetable or animal origin. Bacteria is one of the most studied biological solutions among these biological methods and is one of the most widely used commercial formulations in the world. In this context, biological control instead of chemical control, organic and microbial fertilizer methods are recommended instead of chemical fertilizers (Kotan and Gökçe, 2014).

Microbial fertilizers not only increase the yield and quality of the plant, but also improve the structure of the soil as it dissolves 100%. Thanks to the improved soil structure, the aeration and water holding capacity of the soil increases. It facilitates nutrient uptake by regulating the pH level of the soil. Hairy root formation is accelerated in the plant, which can easily reach the food, and it gains a significant resistance against root diseases. Microbial fertilizers are effective not only for plant growth and development, but also for the multiplication of beneficial microorganisms in the soil. As a result of the combination of all these factors, the importance and necessity of microbial fertilizers has emerged.

Most of the studies on microbial fertilizers are generally Serratia, Pseudomonas, Burkholderia, Agrobacterium, Erwinia, Xanthomonas, Azosprillum, Bacillus, Enterobacter, Rhizobium, Alcaligenes, Arthrobacter. Acetobacter. Acinetobacter. Achromobacter, Aerobacterium, Aerococellabacteria, Azotobacter, Astobacteriaceae, Clostricus Microbacteriaceae and Penicillium fungi (Katırcıoğlu, 2014). Among these genera, especially Pseudomonas and Bacillus are noteworthy due to their stimulating effects on plant growth, as well as their very good antagonistic properties in terms of pathogens (Aşkın et al., 2014). In addition, intensive researches are carried out on the use of some Aspergillus, Penicillium and Trichoderma fungi as biological fertilizers and positive results are obtained. It is also known that these fungi have been registered as microbial fertilizers in Europe. According to the researches, it is estimated that the nitrogen support made to plants

by biofertilization all over the world today constitutes approximately 65% of the total nitrogen amount in the soil (Katırcıoğlu, 2014). As it can be understood from here, the undeniable contribution of biological fertilizers to sustainable agriculture should be seen and their use should be increased. In this context, biological control instead of chemical control, organic and microbial fertilizer methods should be recommended instead of chemical fertilizers.

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#### **CHAPTER 5**

# THE ROLE OF PLANT GROWTH PROMOTING BACTERIA IN ORGANIC AGRICULTURE

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### INTRODUCTION

In recent years, the unconscious use of pesticides and fertilizers has led to the emergence of products that are of poor quality and threaten human health in plant production. Chemicals leaking deep into the soil threaten human and animal health by infiltrating water resources. In addition, chemicals accumulating in the soil affect plant health negatively and disrupt the ecological balance. In our country, increases in chemical inputs have been observed in the last few years. While the total amount of chemical pesticides used in 2010 was 38,555 tons, this figure reached 53,672 tons in 2020. Likewise, the use of chemical fertilizers increased from 9,592,752 tons in 2010 to 14,495,815 tons in 2020 (TUIK 2021). Consumers and producers, who are aware of the negativities of modern agriculture, have turned to agricultural production with methods that do not destroy nature and do not harm human health. For this purpose, Organic Agriculture has emerged as a new agricultural production style. Organic agriculture is an alternative production method that prohibits the use of chemical pesticides, hormones and mineral fertilizers and aims to increase the amount and quality of products without disturbing the ecological balance. Organic agriculture is not only a healthy food production method, but also an effective agricultural method in protecting biodiversity, preventing erosion and, reducing the effects that cause environmental pollution. The relationship between plants and microorganisms is an alternative way to increase crop yield in organic farming system. The use of microbial-based fertilizers without the use of synthetic fertilizers,

which is one of the basic principles of organic agriculture, is increasing in most developed countries. In our country, there has been a slight increase in agriculture with the use of microbial fertilizers in recent years. While organic agriculture production was 1,343,737 tons in 2010, it increased to 1,631,943 tons in 2020 (TUIK 2021). The global biofertilizer market covers various regions of the world such as Europe, Africa, North America, Latin America, Asia-Pacific and the Middle East. The global biofertilizer market is estimated to reach US\$3.5 billion by 2025 (Basu et al., 2021).

Microorganisms in the soil improve soil quality. It also plays important roles in agriculture by promoting plant growth and nutrient uptake. Rhizobacteria that promote plant growth are defined as microorganisms that benefit plants (Igiehon et al., 2019). PGPR supports the growth of plants by ensuring the availability of macro- and micronutrients necessary for the plant using different mechanisms (Kumari, 2018). PGPR promotes plant growth with direct and indirect mechanisms of action. The direct mechanism includes phosphate solubilization and mineralization, production of bio-fertilizers such as ethylene, abscisic acid, auxins and IAA, and nitrogen fixation. The indirect mechanism includes induction of disease resistance, production of secondary metabolites, production of siderophores, and antagonistic activities (Kang, 2012). PGPR also promotes ACC deaminase enzyme production, auxin synthesis, organic acid production, and plant nutrient uptake (Güneş et al., 2015; Caballero-Mellado et al., 2007). Among the commonly known PGPRs Acinetobacter, Allorhizobium, are

Aeromonas, Bacillus, Agrobacterium, , Azoarcus, Azorhizobium, Azospirillum, Paudiacibanase, Azotobacter, , Bradyrhizobium, Burkholderia, Arthrobacter, Caulobacter, Chromobacterium, Delftia, Enterobacter, Enterobacter, Panudiaciba, Enterobacter, P., Rhizobium, Thiobacillus, Serratia, Streptomyces, (Ahemad et al., 2014; Vessey et al., 2003; Parray et al., 2016; Goswami et al., 2016; Kalam et al., 2020; Ahmad et al., 2008).

The use of PGPR inoculants has been studied in detail and the application of suitable bacteria to advance agricultural systems has been investigated (Babalola et al., 2010). This has led to the growth of the crop, the decline of organisms that cause disease and pests (Kour et al., 2020; Kumar et al., 2017). Therefore, biofertilizers applied to plants, seeds and soil are recommended as an alternative to chemical fertilizers. These fertilizers increase plant nutrition by dissolving, breaking down and mobilizing nutrients, thus reducing the use and cost of chemical fertilizers (Singh et al., 2018; Sattar et al., 2019).

#### PGPR as a biofertilizer

High-cost chemicals such as fungicides, fertilizers, pesticides and herbicides cause environmental pollution. The consequences of pollution are mostly seen in heavy metal contaminated crops and groundwater. It has been determined that heavy metals are important for public health as they can be transmitted to humans and cause important health problems such as cancer (Vahidinia et al., 2019). Considering these harms, alternative ways to overcome these problems are being explored. Biofertilizers offer a useful alternative to these

pursuits. PGPR is used as a biofertilizer to increase crop growth and productivity when used as an alternative to complementary or artificial fertilizers. For example, it has been reported that positive effects were observed as a result of using Serratia marcescens, B. polymyxa, , Pseodomonas cichorii, B. Circulans, P. syringae, B. Pantothenticus, P. Putida, and Bacillus thuringiensis as a biofertilizer in different areas (Agbodjato et al., 2018) PGPR, which is used as a biofertilizer in grains such as rice, wheat and corn, which constitute 42.5% of human nutritional needs, offers an alternative way for the sustainability of agriculture (Revees et al., 2016).

According to Beneduzi et al. (2012) observed that plant growth is increased by taking advantage of the antagonistic potential of rhizome fungi and bacteria. Most of the PGPR isolates are found in potatoes, tomatoes, corn, wheat, etc. it has increased the proportion of dry matter, root length and plant height in various products such as (Bhattacharyya and Jha, 2012). According to Islam et al. (2013) reported that Firmicutes and Proteobacteria bacterial strains seriously affect growth parameters such as shoot and root length, biomass in tomato plants. IAA-producing bacteria have a positive effect on the development and elongation of the root system (Shahab et al., 2009). Also P. polymxa, b. megaterium M-3, and B. it has been observed that some types of PGPR, such as cepacia, have a positive effect on quality parameters, increasing efficiency (Yağmur and Güneş, 2021).

## **PGPR** in Nitrogen Fixation

Nitrogen is one of the molecules necessary for the survival of living things. It is one of the important nutrients necessary for the development, productivity and growth of plants. However, even if there is about 78% N<sub>2</sub> in the atmosphere, it cannot be easily used by plants. PGPR fixes this atmospheric nitrogen and converts it into a form that plants can use. PGPR can be fixed to 100 and up to 290 million tons of N<sub>2</sub> per year with biological nitrogen fixation. Thus, it provides N<sub>2</sub> without adverse effects on the environment (Boyer et al., 2004). PGPR can fix nitrogen directly into the soil through the legume-rhizobium relationship or indirectly through secretions. (Naamala and Smith, 2020). Microorganisms that give nitrogen to the soil are examined in two main groups; symbionts and free-living nitrogen fixers. Symbiotic nitrogen fixers are Sinorhizobium, Frankia, Rhizobium, Allorhizobium Azorhizobium, Mesorhizobium, Bradyrhizobium, Achromobacter, Azoarcus and Burkholderia strains (Turan et al., 2016; Babalola, 2010; Pérez-Montaño et al., 2014). Free-living nitrogen fixers are Azoarcus, Azotobacter, Gluconacetobacter, Herbaspirillum and Azospirillum strains (Vessey, 2003).

Studies have shown that PGPR-infused tomatoes result in similar yields, nutrient uptake, and plant growth when compared to the recommended fertilizer rate without the PGPR vaccine. Thus, the cost of chemical fertilizers in agriculture decreased by 25% (Adesemoye et al. 2009). Similarly, it was stated that the PGPR application applied to the maize plant provided a 20% reduction in the recommended

phosphorus and nitrogen amounts without inhibiting the production and growth of maize. It also increased corn yield by 11.7% and biomass production by 17.9% (Sood et al. 2018). Wheat crops, under water restriction A. when treated with brasilense Sp245, they showed greater grain yield, lateral root growth, higher water content, and root hair growth than other untreated wheat (Creus et al., 2004; Creus. et al., 2005). In addition, up to 76% increase in N2 content was observed in peanuts treated with bacteria such as, Pseudomonas, Agrobacterium, Klebsiella and Ochrobactrum compared to untreated peanut plants (Sharma et al., 2016).

## **PGPR** in phosphate solubility

Phosphorus (P) is one of the most important nutrients for plant growth. It participates in many regulations such as metabolism, protein, and nucleic acid synthesis. However, only 0.1% of the phosphorus in the soil can be used by plants (Maharajan et al., 2018). PGPR directly dissolves inorganic phosphorus or aids organic phosphorus flux through microbial conversion. Thus, it helps to strengthen the root system of the plant (Richardson et al., 2011). There are important phosphorus solvent bacteria that can mobilize forms of phosphorus that are difficult to access. These species include Burkholderia, Agrobacterium spp, , Bacillus, Pseudomonas spp, Paenibacillus, Azotobacter, Rhizobium, Enterobacter (Chakraborty et al., 2009; Babalola and Glick, 2012; Raj et al., 2014, Kumar et al., 2014; Tajini et al., 2012; Bidondo et al., 2011; Istina et al., 2015). Among the strong phosphate decongestants, strains belonging to the genus Pseudomonas attract attention (Rodríguez et al.,

1999). Phosphorus biofertilizers help to dissolve phosphate as well as improve  $N_2$  fixation ability. Some plant growth promoting bacteria make Zn, Fe, etc. available. It can be concluded that phosphate-solving bacteria are extremely important in sustainable agriculture, especially in phosphorus-deficient soils (Adedeji et al., 2020).

## PGPR in potassium solubility

The amount of soluble potassium in the soil is generally low, and about 90% of the total potassium is found in insoluble rocks. PGPR has the ability to dissolve potassium (Han and Lee, 2006). Potassium solvent PGPR, *Bacillus mucilaginosus*, *Acidthiobacillus ferrooxidans*, *Bacillus edaphic*, *Paenibacillus Burkholderia spp.*, strains (Liu et al., 2016). Researchers have shown that potassium-dissolving bacteria can be used as a biofertilizer, increasing nutrient intake. Thus, it is possible to reduce the use of chemical fertilizers (Prajapati et al., 2013; Vessey, 2003; Archana et al., 2012).

# PGPR in siderophore production

Iron plays an active role in various metabolic phenomena, such as respiration, photosynthesis, nitrogen fixation, amino acid synthesis. However, the absorption of iron by both plants and microorganisms is not easy. Iron in the Fe<sup>+3</sup> form is the predominant form; however, this form is very poorly soluble. Fe amount to be absorbed by microorganisms is low. Plants and microorganisms need iron, which causes plants and microorganisms to compete for iron. PGPR secretes siderophores to supply iron to iron-deficient soils and to withstand

competition. Siderophore binds to the form of iron (Fe<sup>2+)</sup> that is free in the rhizosphere and prevents the usability of iron by microorganisms and controls the growth of pathogenic microorganisms (Ikram, 1990). Thus, siderophores alleviate the stress caused by high amounts of heavy metals in plants (Braud, 2006). The importance of siderophores on the growth of plants has been emphasized in many different studies (Mandal, 2014; Braud, 2006). For example, mung bean plants inoculated with the siderophore-producing Pseudomonas strain have been found to have a more improved chlorophyll ratio compared to unvaccinated plants (Sharma, 2003). P. putida and P. fluorescens strains are known to secrete siderophores. Some other bacteria, including B. Cereus and Azotobacter vinelandii, are capable of producing siderophores. Therefore, these bacterial strains can be used as plant growth promoters to increase crop yields (Singh, 2015). Bacillus megaterium also has the capacity to produce siderophores, which helps plant growth and reduces diseases (Chakraborty, 2006).

## PGPR in Phytohormone Production and Plant Health

Bacteria are involved in flowering, the development of root systems, germination, shoot elongation, and the production phytohormones (Antar et al., 2021; Sgroy et al., 2009). Auxins and cytokinins are phytohormones produced by bacteria, similar to phytohormones synthesized in plants. It regulates plant hormone levels and supports plant development and growth and activates defensive responses against pathogens (Backer et al., 2018). In addition, some strains of P. fluorescens promote plant growth and are used as seed inoculants to increase productivity (Walsh, 2001).

Auxin hormone is very important for plant development and growth. It has been reported that more than 80% of bacteria living in the rhizospheric environment can secrete and synthesize auxins. Indole Acetic Acid (IAA) is the most concentrated active phytohormone in plants. It is a hormone that can be found in most plant tissues in general (Maheshwari et al., 2015). IAA production by rhizospheric bacteria is common; It can be produced by various bacterial species such as Enterobacter, Aeromonas, Bradyrhizobium, Rhizobium, Burkholderia, Mesorhizobium, Bacillus, Pseudomonas, Azotbacter and Sinorhizobium. (Çakmakçı et al., 2020; Ahmad et al., 2008; Sharma et al., 2016; Celloto et al., 2012). The ability of bacteria to synthesize IAA plays an important role in the regulation of IAA content in roots (Egamberdieva et al., 2015). Kumar et al. (2019) reported that IAA produced by bacteria can also increase biomass production under water stress conditions.

It has been reported in many studies that PGPR improves plant growth and development by releasing phytohormones. Vikram et al. (2007) reported that 30 isolated phosphate-dissolving bacteria were capable of producing both IAA and GA. WPR-51, WM-3 and WPR-42 strains of Azospirillum and Azotobacter have been reported to secrete IAA (Fatima et al. 2009). Karnwal (2009) stated that *P. aeruginosa* and *P. fluorescens* can produce IAA.

Cytokinins are hormones that regulate physiological events related to seed germination, root and shoot growth, flower formation and fruit production, cell division, leaf fall, interactions of plants with pathogens (Egamberdieva et al., 2015; Akhtar et al., 2020). It has been reported that it can increase tolerance under salt stress and support the growth of plants (Kang et al., 2012; Kunikowska et al., 2013). It has been stated that PGPR strains such as Arthrobacter, Azospirillum, Bacillus, and Pseudomonas synthesize cytokinins and have a positive effect on the root system. Cytokinin supports plant growth and is a biocontrol agent that can be used against pathogens (Naz et al., 2009; Maheshwari et al., 2015).

HCN is a secondary metabolite synthesized by rhizospheric bacteria to regulate root metabolism (Schippers et al., 1990). According to Ahmet et al. (2008) reported that among bacteria, Bacillus and Pseudomonas strains have greater potential for HCN production. HCN production by some bacteria may play a role in suppressing the root pathogen. Voisard et al., (1989) reported that P. fluorescens CHA0 has a significant HCN production in the prevention of tobacco disease caused by Thielaviopsis basicola.

# **PGPR** Against Abiotic Stress

Climate change increases the intensity of abiotic stresses such as high temperature and drought, causing significant losses in crops (Lobell and Field, 2007). PGPR supports plant growth by increasing nutrient absorption, releasing antibiotics and producing phytohormones (Sindhu et al., 2020; Lyu et al., 2020). It has been reported that PGPRs can

significantly improve crop yield, as well as increase plant resistance to stresses (Backer et al., 2018; Lyu et al., 2020). Because PGPR promotes plant growth, it can help promote and improve sustainable agriculture and ecological stability (Adedeji et al., 2020).

Plants undergo metabolic disruption under stressful conditions. Restructuring of metabolism is mandatory for antistress agents to resist adverse conditions (Obata and Fernie, 2012). PGPR plays an important role in withstanding osmotic stress caused by drought and high soil salinity levels (Vurukonda et al., 2016). An increase in the relative water content of the leaf was observed in maize inoculated with the PGPR strain. This resulted in its growth and plant resistance under drought (Gou et al., 2015). Rhizobacteria colonize the root zone of plants and increase the plant's tolerance to drought by producing antioxidants, phytohormones and volatile compounds (Vurukonda, 2016). Sarma and Saikia (2014) observed that Pseudomonas aeruginosa supports the growth of Vigna radiate plants under drought stress conditions. Likewise, P. fluorescens improved the growth of Catharanthus roseus by secreting ajmaline under drought stress (Jaleel et al., 2007). Bacillus subtilis, Ochrobactrum pseudogrignonense and Pseudomonas sp. ACC deaminase, produced by , regulates ethylene level and supports drought stress tolerance in plants (Saikia et al., 2018). Some metabolic changes were observed in other agricultural products such as wheat, bell pepper, tobacco, soybean, tomato, cucumber, barley, and lentil under drought and salinity stress in PGPR treated plants (Yasin et al., 2018; Barnawal et al., 2017; Khalid et al.,

2004; Asghar et al., 2002; Dubey et al., 2019; Kidoglu et al., 2008; Cakmakci et al., 2007).

In plants treated with PGPR, it was observed that the proline concentration in shoots increased under salt stress conditions (Younesi, 2014). Arzanesh et al. (2011) noted that wheat germ is treated with Azospirillum spp, which produces IAA, which improves drought tolerance and increases root growth. Gagne-Bourque et al. (2016) found more developed roots and shoots, higher photosynthetic yield, and better stomatal conductivity in B. subtilis-treated Phleum pratense L. compared to the control plant. *Pseudomonas spp.* In a study examining the effects of bacteria on pea plants under drought conditions, it was stated that the plant treated with bacteria showed better grain yield, and pod maturation was delayed. They also reported that the plant's response to drought stress was promising (Arşad et al. 2008). Tiwari et al., (2019) reported that Pseudomonas putida MTCC5279 improved drought stress in chickpea (Cicer arietinum). It has been noted that this positive stress response is induced by bacteria.

The seeds of canola were inoculated with Enterobacter cloacae HSNJ4, which improved the level of plant growth and salt tolerance (Li et al., 2017). It has been reported that the plant inoculated with the PGPR strain producing ACCdeaminase enzyme can survive to some extent in saline conditions (Gamalero et al., 2010). It has been reported that Enterobacter and Pseudomonas producing IAA and ACC-deaminase support cowpea growth under saline conditions (Thuan et al., 2016). Paul et al. (2008) reported that *P. fluorescens* MSP-393 responds to salt stress and facilitates plant growth in a saline environment. It has been reported that antioxidative enzymes containing PGPR facilitate plant growth in the saline environment by removing H2O2 from salt-stressed root regions (Kim et al., 2005). It was observed that *Achromobacter piechaudii* increased the weight of tomato seedlings grown under salt stress and decreased the ethylene level (Mayak et al., 2004). Zafar-ul-Hye et al. (2015) also examined the effects of two *Pseudomonas* species alone on maize plants as well as in combination with manure. As a result of the study, an increase in plant weight and root shoot length was observed in saline environment. Therefore, natural PGPRS can provide an appropriate solution to overcome the challenges of sustainable agriculture, increase crop yields, ensure its quality, and reduce the application of chemical fertilizers (Guerrieri et al., 2020).

# **PGPR** Against Biotic Stress

Biotic stresses are a serious problem in agriculture and cause large crop losses. Changes in temperature and precipitation caused by climate change have led to crop diseases and pests (Naamala and Smith, 2020). PGPR can act as biocontrol agents for plant protection against harmful organisms, including fungi, microorganisms, viruses (Myresiotis et al., 2015; Mishra et al., 2015; Ali et al., 2020). It can also control the external enemies of PGPR, controlling biological stress (Pangesti et al., 2015; Alizadeh et al., 2013). Many studies have found that PGPR application increases plant growth and yield, stimulates systemic resistance, and prevents the use of chemical fertilizers and synthetic pesticides. Moussa et al. (2013) inoculated wheat with *Pseudomonas* 

fluorescens and Bacillus subtilis to reduce the negative effects of Fusarium graminearum on it. Bacillus cepacia reduced the harmful effects of Fusarium culmorum and Fusarium oxysporum on potatoes (Recep et al., 2009). It has been reported that Bacillus amyloliquefaciens Bs006 and Pseudomonas migulae Pf014 reduce the negative effects of Fusarium oxysporum on grapes (Díaz et al., 2013). The strains that affect the growth of *Meloidogyne incognita* are *Bacillus* subtilis (Sneb 815), Pseudomonas fluorescens and Pseudomonas putida (Viljoen et al., 2019; Zhao et al., 2018). Species from Bacillus, Aeromonas, Alcaligenes, Streptomyces, Rhizobium and Pseudomonas are well-documented biocontrol agents with their ability to control plant pathogens (Ahmad et al., 2008; Abdelmoteleb and González-Mendoza, 2020; Das et al., 2017; Alemu, 2016; Zachow et al., 2017;). It has been reported that HCNs secreted by bacteria suppress diseases caused by Macrophomina phaseolina and Meloidogyne javanica. In recent studies, it has been reported that HCN can improve plant growth by affecting plant pathogens (Reetha et al., 2014; Siddiqui et al., 2006).

# Challenges of PGPR production and its use in agriculture

Despite the many benefits, the use of plant growth-promoting bacteria as an alternative to chemical fertilizers is not common due to some difficulties. The most important of these difficulties; Most studies using inoculants are done on a small scale or in the laboratory. Studies in greenhouse and laboratory experiments are not fully reflected in the field. Also, the effects of PGPR are mostly directed at a targeted organism. While chemical fertilizers tend to affect the entire resident microbiota, the target of PGPRs remains specific. Therefore, quality and productivity may not yield the desired results in field conditions (Schütz et al., 2018). In addition, the selection of suitable PGPR strains from thousands of bacteria is a very difficult and demanding process. Selected bacterial strains should not be selective in nature or targeted to particular crops and should exhibit a wide host range. Bacterial strains should be selected according to their performance under different environmental conditions and soil types (Meena et al., 2020). Strains should be effective in replacing unproductive strains and should not antagonize with other beneficial microorganisms in the plant rhizosphere (Mahajan et al., 2009). Therefore, it is necessary to find an appropriate technique for examining and selecting specific PGPR strains in order to perform useful experiments. Bergey's Manual of Determinative Bacteriology is one of the leading methods used to screen new isolates based on morphology and physiology (Compant et al., 2005; Compant et al., 2010).

PGPRs must have essential properties for their use as a successful and efficient bioinoculant. It should be able to live in the soil, be compatible with the grafted crop, interact with the native microflora and abiotic factors in the soil. PGPR to be used as a biofertilizer is grown under a variety of environmental conditions, including varying temperature, soil type, precipitation, and crop diversity. Therefore, a discrepancy can be seen in the efficacy of PGPR-based biofertilizers (Kamilova et al., 2015; Barea, 2015). In addition, the effectiveness of biofertilizers may decrease due to harmful residues of chemicals and adverse conditions

(Mahajan et al., 2009; Parnell et al., 2016). Environmental stresses such as drought and salt in some areas also reduce the biological activities of bacteria (Arora et al., 2010). In addition to these factors, the acidity and alkalinity of the soil, the application of pesticides to the soil can limit its N-fixing ability. Therefore, PGPRs to be used as biofertilizers must be sufficiently colonized in the host plant roots and be able to create a suitable rhizosphere for plant growth. In addition, it should be able to increase the uptake and antagonistic properties of substances such as P. N, K. (Vessey, 2003; Vejan et al., 2016).

Some Gram-negative rhizobacteria exhibit biocontrol potential but are difficult to formulate because they lack the ability to produce spores. In addition, bacteria have formulations that do not have a long shelf life, and after drying they tend to lose their viability (Goswami et al., 2016; Kamilova et al., 2015; Thomas et al., 2019). Difficulties are also encountered in keeping bacteria alive in vaccination. The viability of bacteria may depend on processing methods and storage temperature. For the use of PGPR, it is necessary to maintain its viability at room temperature for a long time (O'Callaghan et al., 2016). Biofertilizers with a short shelf life should be sold or used before the expiry date. Also, since biofertilizers contain live microorganisms, extra care should be taken in their transportation and storage. The product has a high risk of mutating due to technical limitations or problems during storage (Mahajan et al., 2009). Mutations cause a net decrease in the efficiency of the bioinoculant and pose a serious problem that increases the production cost of the bioinoculant. In addition, the insufficient

availability of soil-specific strains on a regional basis significantly limits the widespread use of bio-inoculants. This can result in net monetary loss for companies producing PGPR (Basu et al., 2021).

Registration of a biological or biobased product is a difficult process. The product registration process takes a long time, complex documents are required for registration and high fees are required. Since the laws on registration of biosimilars vary by country, each country has its own laws. It may be difficult for companies to meet the requirements if they want to promote their products in different countries (Basu et al., 2021). In addition, the lack of sufficient and large financial resources in biofertilizer production is a disadvantage (Arora et al., 2010). Once the biofertilizer is produced, the small producers do not have sufficient funds to carry out the distribution on their own. If the distribution is delayed, the quality and biocontrol power of the product deteriorates (Mahajan et al., 2009).

The response of field crops to applied biofertilizers is very slow and it takes time for the vaccine to establish concentration and root colonization. It is difficult for farmers to adopt technologies whose effectiveness is not guaranteed, or to abandon fertilizers and pesticides that have proven effective for more than 50 years. The fact that small doses of chemicals are more effective and rapid in terms of nutrient availability and pathogen control compared to PGPR strains or PGPR-related products further limits the possibility of adoption of PGPR and bio-based products. This causes less preference of biofertilizers by farmers. (Mahajan et al., 2009; Parnell et al., 2016). In addition, farmers

do not have enough information about the advantages of biofertilizers for sustainable agriculture. Therefore, interest in such environmentally beneficial products is declining. The lack of necessary technical support and appropriate equipment, trained workforce and personnel is one of the important infrastructure problems. It is difficult to inform the farmers due to the establishment of extension centers and the lack of trained technical personnel (Mahajan et al., 2009). In addition, the high costs of biocompounds, biofertilizers and biopesticides are a disadvantage. Because bacterial biofertilizers are new to the market, their prices will only decrease when they are widely produced and/or distributed (Basu et al., 2021). All this poses challenges to the use and acceptability of PGPR.

#### **CONCLUSION**

Chemicals, pesticides and herbicides used in agriculture harm the environment. There is a need for alternative plant nutrition and protection products instead of chemical fertilizers that threaten human and environmental health. Meeting this need is possible with the application of organic fertilizers (biofertilizers). While chemical fertilizers harm the soil and environmental health, biofertilizers are natural products and do not pose a threat to the ecosystem. Therefore, fertilizers based on natural products have been an integral component of organic farming to manage long-term soil fertility and maintain crop productivity. In recent studies, it has been determined that PGPR bacteria are significantly effective in increasing the yield and quality parameters of plants grown in organic farming systems. In the future,

there will be a need for cost-effective PGPR products for farmers that provide a suitable growth environment for the plant and are non-phytotoxic for plants, tolerant to adverse environmental conditions, with high quality and high yields.

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# **CHAPTER 6**

# THE EFFECT OF POTASSIUM HUMATE, ITS APPLICATION AND BACTERIAL INOCULATION ON YIELD AND YIELD COMPONENTS IN BEANS

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# **INTRUDUCTION**

Inadequate and unbalanced nutrition is one of the most important problems today. The limited content of some amino acids in cereal protein and the high prices of foods of animal origin have made edible legumes an indispensable alternative in meeting protein needs (Şehirali, 1988). Beans are among the vegetables that have an important place in human nutrition as a legume plant originating from America. It is native to Mexico, Guatemala, Colombia, and Central and South America. Bean (Phaseolus vulgaris L.) seeds have a high protein content of 19-31% (Adams et al., 1985) as well as 61% carbohydrates, 1-2% fat, and are very rich in vitamins A, B and D (Şehirali, 1988). Beans, which are among the edible legumes, have an important place in both world and country agriculture and are widely consumed in Turkey as well as in many countries (Şehirali, 1988).

In addition to being consumed fresh, dry and canned, the high nutritional value of beans increases the importance of beans. In addition, in developing countries, it is among the indispensable types of vegetable gardens because it constitutes the main consumption item. Beans bring the nutrients in the lower layers of the soil to the soil surface through its developed root system and fix nitrogen to the soil it grows through the nodules formed by the Rhizobium phaseoli bacteria in its roots (Sprent and Sprent, 1990). Therefore, it is one of the most important cultivated plants that should be alternated in irrigated farming areas, as it meets the nitrogen need of the next plant and constitutes an important plant group in terms of crop rotation (Adams et al., 1985).

Beans are the most widely grown edible legumes in the world. The bean plant, which has a wide adaptation area from 520° north latitude to 320° south latitude in the world, is produced in areas close to sea level in America and Europe, and in areas higher than 3000 m in South America (Graham and Ranalli, 1997).

Beans are the most selective edible legumes in terms of ecological conditions. Physical (precipitation, temperature, topography, soil type, etc.), biological (diseases and pests) and socioeconomic factors affect bean cultivation, yield and quality in a region (Woolley et al., 1991). Although the bean is a temperate climate plant, it is very sensitive to frost damage. In addition, the soil temperature must be above 15 oC for successful germination, rapid and simultaneous emergence. Germination and emergence will be prolonged at low temperatures, and the probability of damage by abiotic or living factors such as soil drying increases during this period. In late sowing, especially in hot summer months, the germination rate decreases due to high temperature, seedling growth decreases (Lin and Markhart, 1996), flowering and pod binding are negatively affected (Scully and Waines, 1988), or when planting is done later, autumn precipitation with the first frosts will be harmful.

Humic acids, one of the most important organic soil conditioners, are black and dark brown colored substances formed as a result of natural deterioration of plant and animal residues. Humic acid is found in soil, animal manure, peat beds, leonardite and lignite. The most active ingredient of humus, which contains 30-35% protein

carbohydrates, 40-45% lignin and other minerals, is humic acids. In addition, since humic acid has a higher cation exchange capacity than all organic fertilizers, it ensures the highest absorption of nutrients. Humic acid applications are considered as the most effective way to provide the soil with vital macro and micro nutrients naturally (Anonymous, 2007).

Considering the agricultural importance of humic acids, since it improves the soil texture and structure physically, the soil gains easy-to-work and soft characteristics thanks to this substance, and it also creates a permeable and soft soil structure by breaking down compacted loam and clayey soil. While humic acid increases the water holding and respiration ability of the soil, it increases the germination level of the seed and provides space for the recovery of the microflora population in the soil and colonization (Bhardwaj and Gaur., 1971). Humic acid increases the organic matter level of the soil, making it easier for micro and macro nutrients to reach the plant. When evaluated in general, it can be stated that humic acids act as a bridge between the nutrients in the soil and the plant.

Humic acid has positive effects on plant roots and gives better results for the growth and development of plants. It stimulates the H-ATPase enzyme activity in the stem cells of plants and promotes their nutrient and water intake.

The energy released as a result of the oxidation of organic carbon protects the plant against cold extreme conditions by keeping the root

zone of the plant warm. In addition, some types of fungi, which are formed as a result of the biological activities of microorganisms in the soil, enable the reproduction of natural antibiotics and their release into the soil, thus giving the plant resistance (Özkan, 2007). The use of organic fertilizers such as humic acid, which is beneficial for both plant health and soil texture, and applications to increase the yield of cultivated plants are of great agronomical importance.

Legumes have the ability to bind the elemental nitrogen of the air to the soil through Rhizobium bacteria living in their roots. As a result of this event, called biological nitrogen fixation, the soil layers in which legume roots spread are largely fertilized with organic nitrogen (Şehirali, 1988). It is accepted that approximately 25% of the Rhizobium bacteria population naturally found in the soil are effective in biological nitrogen fixation. In order to increase this rate, seeds should be inoculated with specially prepared bacterial cultures (Pekşen and mutlur, 1996). Soil moisture has a significant effect on the survival and reproduction of Rhizobium bacteria added to the soil by grafting. Too little or too much moisture in the soil causes the bacteria to disappear to a great extent (Sepetoğlu, 1992).

This study was carried out to determine the effect of humic acid applied to the seed at different doses and bacterial inoculation on yield and yield components of beans grown in Bitlis-Ahlat conditions.

#### MATERIAL and METHOD

#### Material

The research was carried out under irrigated conditions in a farmer's field in Adilcevaz district of Bitlis province. Karacaşehir-90 variety was used as material in the research. Karacaşehir-90 bean variety is dwarf, early, tolerant to virus and bacterial diseases, its grain color is white and its grain shape is small plump.

Rhizobium bacteria used in the experiment were obtained from the Soil Fertilizer and Water Resources Central Research Institute. Humic acid used in the research was used in the form of Potassium Humate. In the experiment, Control (PH0) was used as Potassium Humate application and 100g (PH1), 200g (PH2), 300g (PH3), 400g (PH4) Potassium Humate was used as application to 100 kg seed.

#### Location of the research site

The town of Adilcevaz, where the research was conducted, is located in the Eastern Anatolia Region and is connected to the province of Bitlis. It is located on the shore of Lake Van. It is located in a basin surrounded by mountains at the foot of Mount Süphan. The altitude of the district is 1676 m and it is located at 380 48' north latitude and 420 44' east longitude. The trial area is approximately 400 m from Lake Van.

# Climatic characteristics of the research site

Continental climate prevails in Adilcevaz district. The winter season is cold and covered with snow, and the summers are cool and dry. Due to

the fact that the district is located on the shore of Lake Van, it is milder than the inner parts with the positive effect of the lake. The fact that the soil surface is covered with snow during the winter months is an important factor in reducing the cold damage in winter plantings. The climate data of the months covering the period of the experiment and the long-term average are given in Table 3.1. The annual precipitation amount of the region where the research was conducted is 566 mm, the average temperature is 8.7 °C, and the average relative humidity is 48.7%, regarding the long-term average in the growing season. The amount of precipitation falling in the 2016 growing season is 101.9 mm. The average temperature is 9.5 °C, and the average relative humidity is 45.4% (Anonymous, 2016).

Table 1. Long-term average and some climate data of 2016 in Adilcevaz district of Bitlis province

	Precipitatio (mm)	n	Avg. temp	).	Relative	humidity (%
Months	2016	UYO	2016	UYO	2016	UYO
May	68.0	45.5	12.1	13.0	59.5	56.0
June	29.0	17.7	16.7	18.1	45.9	50.0
Jully	7.0	5.5	21.2	22.2	39.6	45.0
August	6.0	13.0	21.1	17.2	36.7	44.0
Total	101.9	81.8				
Average			19.5	6	45.4	
· ·			17.		48.75	

#### Soil characteristics of the research site

Some physical and chemical analyzes of the soil samples taken from different depths of the soil where the experiment was established were made in the Fertilizer Soil Analysis Laboratory, and the analysis results are given in Table 3.2.

	Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture Class	Hd	Lime (%)	Salt (%)	Org. Matter (%)	Total % N	P (ppm.)	K (ppm.)
(	0-20	27.1	34.0	39.9	Clayey-Loam	8.3	18.1	0.022	1.86 0	.093	6.72	562.12
1	20-40	30.1	32.5	37.4	Clavev-Loam	8.0	13.9	0.020	1.82.0	.087	4.23	223.23

**Table 2.** Some physical and chemical properties of the soils of the trial area (Gübretaş Soil Analysis Laboratory results)

According to the results of the soil analysis, it is seen that the soils of the trial area have a clayey-loam texture and the lime content is high. While the soils are in the good group in terms of salt content, their pH was determined as slightly alkaline reaction.

Organic matter and nitrogen content were found to be very low in all layers of the soil. The available phosphorus content is also very low, potassium is high at 0-20 cm, and it is determined to be at a sufficient level as you go down to the lower layers.

#### Method

The experiment was carried out in three replications according to the divided plots experimental design in randomized blocks in 2016. Bacteria inoculation was placed in the main plots (Inoculation-Uninoculation), and Potassium Humate doses (Control, 100, 200, 300 and 400 gr/100kg seeds) were placed in sub plots. Potassium Humate doses were applied to the seed one day before and planting was done after the seed was dried. A total of 30 plots were used in the experiment. Each plot was set to 4 rows, the row spacing was set as 10 cm and the

spacing between rows was 50 cm. There is a 2 m gap between the parcels and 2 m between the blocks. The parcel area; It is arranged as  $2.0 \text{ m x 4 m} = 8.0 \text{ m}^2$ . The amount of seed planted in the plot, corresponding to 20 seeds per square meter, was determined (Sehirali, 1988). At harvest, the 4 rows forming the parcel, one row on each side, and the plants within 50 cm of the parcel heads were excluded as an edge effect. (Ceylan and Sepetoglu, 1979). Measurements and weighings were made over an area of 1.0 m x 3 m =  $3 \text{ m}^2$ . Sowing. harvesting and threshing were done by hand. The seeds were inoculated with a bacterial culture containing 10<sup>6</sup> cells/g one day before sowing, according to Vincent (1970).

# **Cultural practices**

In the autumn of 2015, the trial area was deeply plowed. In the spring of 2016, a second outcrop and then a disc harrow were duplicated and the seed bed was made ready for planting. Sowing was done manually by making lines with a marker.

Weed control in the experimental area was carried out twice, before and after flowering. Harvest was done on 09.09.2016. Measurement, counting and threshing processes of the harvested plants were done meticulously in the laboratory and the average values were taken. Parcel yields, on the other hand, were calculated by threshing by pounding after drying in bundles in the laboratory. The experiment was carried out under irrigated conditions, and it was irrigated as needed with a sprinkler irrigation system, taking into account the precipitation, air temperature and humidity in the soil.

# **RESULTS**

The averages of the effects of inoculation practices and potassium humate doses on some vegetative and yield components of the bean plant are given in Tables 1.

# **Plant Height**

As seen in Table 1, bacterial inoculation and potassium humate applications affected plant height. While the effect of inoculation applications on plant height is not statistically significant, the effect of potassium humate applications on plant height is statistically significant at the 1% level. Among the inoculation applications, the highest plant height was obtained from the application with 51.24 cm, the lowest value was obtained from the application uninoculation with 51.00 cm. When the doses of potassium humate applications were compared, the highest plant height was determined with 57.80 cm from PH4 application, and the lowest value was determined from 41.88 cm from PH0 application (Table 1 and Figure 1).

It was determined that potassium humate x inoculation interaction was statistically significant in terms of plant height criterion. The longest plant height value was determined as 60.50 cm in Inoculation -PH4 application, and the shortest plant height value was determined as 40.57 cm in Uninoculation -PH0 application (Table 1).

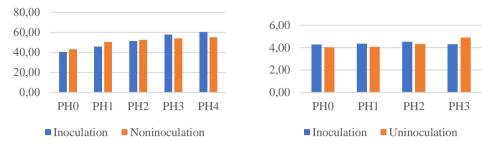


Figure 1. Effect of bacterial inoculation and potassium humate doses Figure 2 The effect of bacterial inoculation and potassium humate doses on the plant height on the branch in the plant

# **First Pod Height**

As seen in Table 1, inoculation and potassium humate applications affected the first legume height in the study. These differences in first pod height were statistically insignificant at the level of 5% between inoculation and potassium humate applications. The highest first pod height was determined from the bacteria inoculation with 8.92 cm, while the lowest first pod height was obtained from the application without bacteria inoculation with 8.52 cm. In terms of potassium humate applications, the average first pod height values in beans varied between 8.35-9.00 cm. The highest value for the first pod height was determined in the PHO application, and the lowest value was determined in the PH4 application (Table 1).

Table 1. PH, FPH, NBP, NPP, NGP, GYUA, HI, BY and HSW and mean values of bacteria inoculation

and potassium humate applications in beans and Duncan groups \*

Bacteria	с аррисацонз н		Potassium Hu			
Inoculation	PHO	PH1	PH2	PH3	PH4	Mean
		Plant Hei	ght (cm) *;**			1
Uninoculation	40.57 f	50.33 d	52.33 cd	54.07.cd	55.10 c	51.00
Inoculation	43.20 ef	45.90 e	51.37 d	57.87 b	60.50 a	51.24
Mean	41.88 D	48.11 C	51.85 B	55.96 A	57.80 A	
		First Pod H	eight (cm) *;**			ı
Uninoculation	9.07	8.40	9.02	8.13	8.00	8.52
Inoculation	8.93	9.43	7.77	9.80	8.70	8.92
Mean	9.00	8.91	8.39	8.96	8.35	
	Number of	Branches per	Plant (Number	r /Plant) *;**		
Uninoculation	4.03	4.07	4.33	4.90	4.70	4.41
Inoculation	4.30	4.37	4.53	4.03	4.43	4.33
Mean	4.16 B	4.21 B	4.43A	4.46 A	4.56 A	
	Numb	er of Pods per	Plant (Numbe	r /Plant) *;**		
Uninoculation	20.03	20.43	22.60	23.60	27.93	22.92 B
Inoculation	23.57	22.43	24.03	27.57	25.47	24.61 A
Mean	21.80 B	21.43 B	23.31 B	25.58 A	26.70 A	
	Number	r of Grains pe	er Plant (Numb	er/ Plant) *;**		
Uninoculation	70.12	71.52	79.10	82.60	97.77	80.22 B
Inoculation	84.84	78.52	84.12	96.48	89.13	86.14 A
Mean	76.30 B	75.01 B	81.60 B	89.54 A	93.45 A	
	G	rain Yield per	· Unit Area (kg	/da) *;**		•
Uninoculation	117.55 d	135.51 d	150.22 cd	209.22 ab	202.44 b	162.90 B
Inoculation	166.89 с	223.55 a	209.55 ab	241.33 a	225.77 a	213.41 A
Mean	142.22 C	179.33 B	179.88 B	225.27 A	214.10 A	
		Harvest	t Index (%)*;**	k		
Uninoculation	32.00	33.67	35.00	36.67	36.00	34.67 B
Inoculation	39.00	39.67	39.67	40.00	42.00	40.06 A
Mean	35.50 B	36.66 AB	37.33 AB	38.33 A	39.00 A	
		Biological	Yield (kg/da) *	**		
Uninoculation	282.22 d	335.77 bc	342.44 bc	322.89 cd	355.55 b	327.37 B
Inoculation	345.78 bc	440.44 a	346.22 bc	540.89 a	460.55 a	426.77 A
Mean	313.99 D	388.10 B	344.33 C	431.88 A	407.05 AB	
		Hundred	Grain Weight	(g)		
Uninoculation	30.93	30.33	30.98	32.85	31.21	31.25
Inoculation	32.57	30.86	30.51	34.19	31.27	31.88
Mean	31.75	30.59	30.74	33.52	31.23	

<sup>\*:</sup> PH: Plant height, FPH: First Pod Height, NBP: Number of Branches per Plant, NPP: Number of Pods per Plant NGP:Number of Grains per Plant, GYUA: Grain Yield per Unit Area, HI: Harvest Index, BY: Biological Yield, HGW:Hundred Grain Weight

<sup>\*:</sup> The mean in the same column, expressed with lowercase letters and indicated with different letters, is statistically different from each other according to Duncan's test within the error limits of P≤0.05.

<sup>\*\*:</sup> The mean in the same column, expressed with capital letters and shown with different letters, is statistically different from each other according to Duncan's test within the error limits of P≤0.05.

# **Number of Branches per Plant**

When the average values of the number of branches per plant are examined from Table 1, it will be seen that the highest value (4.41 units/plant) in terms of inoculation applications occurs in the uninoculation application, and the lowest value (4.33 units/plant) occurs in the application with inoculation. But the difference between the two applications was statistically insignificant. The difference in potassium humate applications was statistically significant at the 1% level. The highest number of branches was determined from PH4 application with 4.56 units/plant, and the lowest number of branches was determined from PH0 application with 4.16 units/plant (Table 1). It has been determined that the interaction of potassium humate x inoculation is important in terms of the number of branches in the plant. While the highest number of branches was determined from the Inoculation-PH3 application with 4.90 units/plant, the lowest value was obtained from the Uninoculation-PHO application with 4.03 units/plant (Table 1, Figure 2).

# **Number of Pods per Plant**

In inoculation applications, which are compared in terms of its effects on the number of pods in the plant, it is seen that the inoculation application has a higher number of pods in the plant than the uninoculation application. The highest number of pods in the plant was obtained from the Inoculation application with 24.61 units/plant, while the lowest was obtained from the Uninoculation application with 22.92 units. When potassium humate doses were compared in terms of the number of pods per plant, the highest number of pods per plant was determined from PH4 (26.70 units/plant) and PH3 (25.58 units/plant) applications, while the lowest pod number was determined from PH1 application with 21.43 units/plant (Table 1). The interaction between potassium humate x inoculation applications was found to be insignificant in terms of the number of pods per plant.

# **Number of Grains per Plant**

When the grain number values in the plant in Table 1 are examined, it is seen that the inoculation application (86.14 units/plant) has a higher effect than the uninoculation application (80.22 units/plant). Among the potassium humate applications, the highest number of seeds per plant was obtained from PH4 with 93.45 units/plant and PH3 with 89.54 units/plant. This difference was statistically significant at the 1% level (Table 1).

# Grain Yield per Unit Area

When the inoculation application averages were compared in terms of grain yield per unit area, the inoculation application had the highest grain yield per unit area with 213.41 kg/da, while the lowest unit area grain yield was 162.90 kg/da uninoculation. When the mean potassium humate doses are compared, it will be seen that the highest grain yield per unit area was determined from PH4 (214.10 kg/da) and PH3 (225.27 kg/da) applications, and the lowest unit area grain yield was determined from PH0 (142.22 kg/da) applications (Table 1, Figure 3).

Inoculation x potassium humate relationship was found to be statistically significant in terms of grain yield per unit area. While the highest value was determined as 241.33 kg/da from Inoculation-PH3 application; the lowest value was determined as 117.75 kg/da in the Un inoculation-PHO application (Table 1, Figure 3).

# **Biological Yield**

As seen in Table 1, when Inoculation application averages are compared in terms of biological yield, it is seen that Inoculation application (426.77 kg/da) has more biological efficiency compared to Uninoculation application (327.37 kg/da). When potassium humate doses were compared, the highest biological efficiency was obtained from PH3 (431.88 kg/da) application, and the lowest biological efficiency was obtained from PH0 (313.99 kg/da) application (Table 1, Figure 4).

When Table 1 and Figure 4 are examined, it is seen that the biological yield averages of the inoculation x potassium humate interaction vary between 282.22 - 540.89 kg/da. With a biological yield of 540.89 kg/da, the maximum value was obtained from the Inoculation-PH3 application, and the minimum value as 282.22 kg/da was obtained from the Uninoculation-PH0 application.

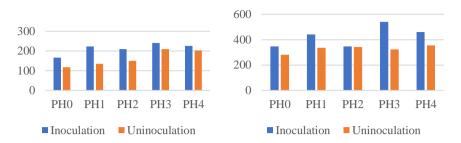


Figure 3. The effects of bacteria inoculation and potassium humate potassium humate doses grain yield per unit area

Figure 4. Effect of bacterial inoculation and doses on biological yield

#### **Harvest Index**

When Table 1 is examined in terms of harvest index, it is seen that inoculation practices have a positive and significant effect on the harvest index, while the effect of potassium humate applications is statistically insignificant. Among the inoculation applications, the highest harvest index was 40.06% in the inoculation application, and the lowest in the Uninoculation application with 34.67%. When the doses of potassium humates are compared, it is seen that the highest harvest index was determined from PH4 (39.00%) and PH3 (38.33%) applications, and the lowest harvest index was determined from PH0 application with 35.50% (Table 1).

# **Hundred GrainWeight**

In the study, inoculation practices did not have much effect on hundred grain weight. While the highest average value was obtained from the inoculation application with 31.88 g, the lowest average value was obtained from the uninoculation application with 31.25 g. When the

averages of Potassium Humate applications were compared in terms of hundred grain weight, the highest value was determined from PH3 (33.52 g) and the lowest value from PH1 (30.59 g) application (Table 1).

#### **DISCUSSION**

Significant differences were found in terms of the characters examined in the study. Depending on the increase in potassium humate doses, increases in growth and yield related properties were achieved. Organic materials have a positive effect on the physical, biological and chemical properties of the soil. This positive effect also affects the growth and development of plants and causes an increase in yield. Humic substances not only affect the microbiological, physical and chemical properties of organic substances in the soil, but also have a direct effect on plant growth (Chain and Avaid, 1990).

Patil et al. (2011) reported that potassium humate application increased plant height compared to control. Bozoğlu et al. (2004) reported that potassium humate application increased the number of pods in the plant and it was statistically significant. In addition, researchers reported that humic acid caused an increase in the number of pods in the chickpea plant (Ünsal 2007) and the number of grains in the plant (Elkatmıs and Togay 2017). Azarpour et al. (2011) reported that they obtained the highest grain yield from 50 mg/l humic acid application, DongFang et al. (2002) stated that potassium humate applied to the soybean plant from the leaves increased the yield by 14.06% on average compared to

the control. Mtua (2005) stated that the increase in thousand grain weight, harvest index and protein ratios in bean was insignificant in parallel with the increase in doses of TKI-Humas application, while it was important in other yield criteria. Casenave de Sanfilippo et al. (1990) also found that humic acid applications had a positive effect on the development of plants. Humic acid can cause an increase in the protein ratio in the grain. Rather, this may have resulted from the improving effect of the current state of the soil. In such cases, the activities of both bacteria show a positive trend. Depending on this situation, nodule formation and nitrogen fixation are encouraged, allowing plants to take more nitrogen from the soil through their roots. Yetim and Yalçın (2008) determined in their study that increasing amounts of nitrogen and humic acid added to the soil increased the protein ratio in beans. Nadir Baloach et al. (2014) stated that the harvest index increased by about 22% compared to the control in a field study where they applied 10 kg of humic acid per hectare. In addition, Massoud et al. (2013) reported that the highest harvest index was obtained from the application that gave 75% humic acid. Putintsev and Platonova (1991) stated that the hundred grain weight increased with the application of humic substances. The data obtained in terms of the characteristics examined from the vaccinated applications resulted in a significant increase compared to the non-vaccinated control. Atmospheric nitrogen obtained by the plants as a result of the grafting application encouraged vegetative development, and as a result, more branches and leaves were formed as well as an increase in plant height. The height of the first pod in legumes varies according to earliness and

plant height (Akçin, 1988). However, there may be slight changes in these values with practices such as cultural and bacterial inoculation. As a matter of fact, it has been reported that bacterial inoculation in beans significantly affects plant height and first pod height (Bildirici, 2003). While Sryvastava and Varma (1986) reported that inoculation applications increased the number of pods per plant in peas, it was stated that the highest seed yield per unit area and the number of pods per plant were obtained in the applications (Ayanoğlu, 1989). In addition, Önder (1992), Karahan (1997), Karahan and Şehirali (1999) and Erman et al. (2009) detected significant increases in the number of pods per plant and the number of grains per plant by inoculation with bacterial culture compared to the control treatment. In some studies, researchers reported that they detected significant increases in grain yield per unit area of plants inoculation (Sehirali et al., 1983, Bozoğlu et al., 1997, Önder and Özkaynak, 1994). High biological productivity is closely related to optimum growing conditions. While the number of pods and grains in the plant can significantly affect the biological yield, the vegetative parts of the plants that find the appropriate growth environment develop better. It has been reported by many researchers that the biological efficiency of inoculation will increase compared to control (Ceylan and Sepetoğlu, 1979, Hoque and Haq, 1994, Kumar et al., 1993, Kantar et al., 1994 and Nadir Baloach et al., 2014). Kaya (2000) reports that bacterial inoculation does not make a significant difference in terms of the average harvest index. Bildirici (2003) reported that the harvest index value was positively and significantly affected by bacterial inoculation.

Eken (2003) and Kaya (2000) stated that bacterial inoculation increased the hundred-grain weight. Karuç (1992) reported that the hundred-grain weights obtained from the applications with bacterial inoculation were higher than the applications without inoculation.

#### **CONCLUSION**

In this study, it was aimed to determine the most appropriate Potassium Humate dose in inoculation-uninoculation conditions in Karacaşehir-90 bean cultivar.

In the study, the effects of Potassium Humate fertilization on plant height, first pod height, number of branches per plant, number of pods and seeds per plant, number of pods per pod, grain yield per unit area, harvest index, biological yield, and hundred-seed weight of beans were investigated in inoculation and uninoculation conditions detected.

In terms of grain yield per unit area, the highest yield was obtained from Inoculation-PH4 application with 223.88 kg/da, while the lowest value was obtained from Uninoculation-PH0 application with 117.55 kg/da. While the first pod height and hundred-seed weight were not affected by inoculation and potassium humate applications, plant height and branch number were not affected by inoculation, and the harvest index was not affected by potassium humate application. In terms of other properties, they were significantly affected by inoculation and humic acid applications.

As a result, considering the other characteristics discussed in the study, 400 g potassium humate per 100 kg seed and bacterial inoculation are considered suitable for bean cultivation, but it is thought that it would be beneficial to repeat such studies in order to obtain better results.

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### **CHAPTER 7**

# THE EFFECTS OF YIELD, QALITY AND ENVIRONMENT OF RHIZOBIUM INOCULATION AND DIFFERENT VERMICOMPOST DOSES ON BROAD BEAN (Vicia faba L.) IN FETHIYE CONDITIONS\*

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#### INTRODUCTION

The objectives of organic agriculture are to enrich biodiversity, biological cycle and biological activity in the soil in order to ensure the social, ecological and economic sustainability of natural systems. Organic matter plays an important role in the vitality and fertility of the soil. The effect of organic matter can be direct or indirect. Organic matter plays a direct role as a plant nutrient source, and plays an indirect role by affecting the physical and chemical properties of the soil. Excessive chemical fertilization used in agriculture causes the decrease of some nutrients in the soil and the excessive accumulation of others. The best way to keep the organic matter in the soil at the maximum level is organic fertilization (Son et al. 2004).

Vermicompost is the product obtained by converting organic materials into a humus-like substance with the help of earthworms (Garg et al. 2010). Vermicomposting is the name given to the decomposition process of waste under suitable conditions. As a result of vermicomposting, a material with fine texture, high porosity, aeration, drainage, water holding capacity and microbial activity is obtained (Garg et al. 2010). Vermicompost application shows that nutrients to be used by plants are formed and it increases the uptake of these nutrients by the plant (Nagavallemma et al. 2004, Peyvast et al. 2007).

It is produced with hygienic production techniques that vary according to the vermicompost cultivation techniques and the characteristics of the earthworms, contain an average of 1.5-2% N, 2.5-4.1% P2O5 and 1.4-9.2% K2O, do not cause health problems, do not contain harmful

microorganisms and heavy metals, and increase the production potential of the soil. are fertilizers (Bellitürk, 2016). Vermicompost is extremely important in terms of plant growth and soil regulating effects, as well as allowing various (vegetable, animal, industrial, etc.) wastes to be used in production (Ceritoğlu et al., 2019).

Edible grain legumes are in the Fabaceae family (Zirek and Toğay 2021). Legumes have the ability to bind the elemental nitrogen of the air to the soil through the Rhizobium bacteria living in their roots. As a result of this phenomenon called biological nitrogen fixation, the soil layers in which legume roots spread are largely fertilized with organic nitrogen (Şehirali, 1988). It is accepted that approximately 25% of the Rhizobium bacteria population naturally found in the soil are effective in biological nitrogen fixation. In order to increase this rate, seeds should be inoculated with specially prepared bacterial cultures (Pekşen and Gülümser, 1996). Soil moisture has a significant effect on the survival and reproduction of Rhizobium bacteria added to the soil by grafting. Too little or too much moisture in the soil causes the bacteria to disappear to a great extent. (Sepetoglu, 1992). Inoculation of plant growth promoting rhizobacteria (PGPR) reduces the harmful effects of salinity on plants (Yılmaz and Kulaz, 2019). Kulaz et al. (1997) found that nitrogen, phosphorus and bacteria inoculation on lentil varieties of Malazgirt 89 increased the yield elements of the plant in the plots where nitrogen and phosphorus were not applied.

The broad bean, which has an important place in the nutrition of people, is consumed in different ways in many countries due to the richness of the vegetable protein it contains.Broad bean seeds are an important protein source in human and animal nutrition with their high protein ratios ranging from 25-35% (Nachi and Guen, 1996). The amount of protein in green pods is 5-7%, and in green seeds in milk production, it is 3-10%. It has many benefits in human nutrition, soil fertility and physical properties, and agricultural production.

Broad bean ranks fourth after lentils, chickpeas and beans in terms of cultivation area and production amount among edible legumes in Turkey. According to 2019 data in our country, the broad bean cultivation area is 4332 ha, the production is 12,346 tons and the yield per decare is 285 kg/da, and it is mostly grown in the Aegean, Marmara and Mediterranean regions. According to 2019 data in the world, the broad bean cultivation area is 2577201 ha, the production is 5.431.503 tons and the yield is 210.7 kg/da (Anonymous 2021).

It is a well-known fact that increasing crop production and income of farmers depends on increasing productivity, and using the right fertilizer is one of the most effective ways to improve productivity. Although the share of fertilizers in the productivity increase varies according to the conditions, it is generally stated that it is around 50%.

Apart from its economic dimension, inorganic fertilizers, which are widely and unconsciously used, cause significant damage to the plant and the environment, especially the soil. It takes a long time to repair these damages and sometimes it is not possible to compensate. The fact that the chemical inputs used within the framework of efficiency

improvement efforts are at a level that threatens health caused the consumer preference to change again. In this context, the organic farming system, which increases food safety, has been developed within the scope of agricultural production.

In organic plant production, the supply of nutrients needed by plants largely depends on soil fertility. Soil fertility is provided by applications such as organic worm manure, compost, green manure and farm manure. Due to its rich content, vermicompost provides high added value in sustainable agriculture and organic agriculture. Vermicompost is a remedy for pollution caused by other pollutants. Worm fertilization, modern agricultural practices are important in terms of preventing negative consequences such as environmental pollution, decrease in soil organic matter, and salinity. Vermicompost production is a "sustainable" method for the "domestic and industrial waste/waste" problem, which is an environmental problem that grows with the level of urbanization and industrialization.

In this study, it was aimed to compare the effects of different doses of vermicompost on the yield, some yield elements and the environment, in Fethiye conditions, with and without bacteria inoculation, on the Salkım broad bean variety. It is aimed to examine the possibilities of using the methods that can be used.

#### MATERIAL AND METHOD

#### Materiel

In the study, Salkim broad bean variety was used as plant material. The average number of seeds per pod of this variety is 3-4, and the weight of one hundred grains is 132-154 g. and its average yield is 355-448 kg/da, and it is also resistant to -8.6 degrees.

In the study, vermicompost (2.4% N, 1.4% P, 1% K) fertilizer was given to the broad bean plant as an organic fertilizer source. At the same time, the grafted cultivars were Rhizobium leguminosorum biovar. viceae bacterial culture was contaminated.

#### Climatic characteristics of the research site

In the Fethiye District of Muğla Province, the winter season is rainy and mild, and the summers are dry. Mediterranean temperate climate prevails. The climate data of the months covering the period in which the experiment was carried out and the long-term average are given in Table 1. The annual precipitation amount for the long-term average of the growing season of the region where the research was carried out is 885.1 mm, the average temperature is 15.2 °C, and the average relative humidity is 66.6%. The amount of precipitation falling in the 2018-19 growing season is 961.2 mm, the average temperature is 16.0 °C, and the average relative humidity is 67.2% (Anonymous, 2019).

Table 1. Muğla province Fethiye district long-term average and some climate data for the 2018-2019 growing season (Anonymous 2019)

	Preci	pitation	Av. T	Av.		
	(mm)		Relativ			
Months	2018-19		2018-19	LTA	LTA 2018-19	
	LTA					
November	121.1	120.3	15.0	14.7	72.2	66.5
December	198.4	179.1	11.7	11.3	73.2	70.6
January	304.6	176.8	10.3	10.1	75.1	70.4
February	177.9	123.9	12.1	11.0	71.3	67.7
March	91.8	82.3	14.1	13.2	65.0	67.0
April	54.8	48.1	16.5	16.3	63.7	66.6
May	4.2	27.9	21.5	20.4	59.1	65.1
June	8.4	6.4	26.8	24.9	57.6	59.4
Total	961.2	885.1				
Avarage			16.0	15.2	67.2	66.6

### Soil characteristics of the research site

According to the results of soil analysis of soil samples obtained from 0-20 cm depth of the study area, clay loamy textured, slightly alkaline reaction, organic matter content was found to be medium, lime content was found to be slightly calcareous, unsalted, phosphorus content was moderate, and potassium content was sufficient. Some physical and chemical analyzes of the soil samples of the field where the experiment was set up were made in Fetlab Analysis Laboratory, and the results of the control soil analysis are given in Table 2, and the soil analysis results taken from the same areas after the study was completed are given in Table 3. In Table 3, it is observed that organic fertilizers applied in pH, phosphorus, lime, potassium, organic matter and total salt values are improvements.

Table 3. Some physical and chemical properties of experimental area control soils

Depth	Texture	pН	Lime	Phosphorus	Potassium	OrganicM	Total Salt
( cm )		(1:2.3)	(%)	(ppm)	(ppm)	atter	(%)
						(%)	
0-20	Clay Loam	7.96	2.536	12.252	109.35	2.169	0.0404

Table 4. Some physical and chemical properties of the parcels applied vermicompost in the experimental area

Depth	Texture	pН	Lime	Phosphorus	Potassium	OrganicM	Total Salt
( cm )		(1:2.3)	(%)	(ppm)	(ppm)	atter	(%)
						(%)	
0-20	Clay Loam	7.21	2.124	12.410	112.78	2.786	0.0301

#### Method

In the study, Salkım broad bean variety was used as plant material. In the study, vermicompost (2.4% N, 1.4% P, 1% K) fertilizer was given to the broad bean plant as an organic fertilizer source. The trial was conducted in Fethiye in 2018-19. The experiment was carried out in randomized blocks according to the split plot design with three replications. There are 24 parcels in the experiment. Each plot consists of 5 rows; the row spacing of the parcels is 45 cm. parcel area; It is planned to be  $1.8 \text{m x } 4 \text{m} = 7.2 \text{m}^2$ . The amount of seed to be planted in the parcel was determined so that 50 seeds per square meter. Before planting the seeds to be inoculated with bacteria, Rhizobium leguminosorum biovar. viceae bacteria culture was contaminated. Vermicompost was applied at doses of 0 kg/da, 250 kg/da, 500 kg/da and 750 kg/da. All the operations were carried out on 1.35 m x 3 m = 4.05 m<sup>2</sup> areas, leaving the plants within 50 cm of each side of the 5 rows forming the parcel and 50 cm from the tops of the rows out of the observation as an edge effect. The sowing process was carried out on November 15, 2018. Fresh weights were calculated by counting the nodules formed on the roots during the flowering period. The effects of vermicompost on the soil and the environment were investigated by making soil analyzes.

In the autumn of 2018, crowbar plow was applied to the trial area, then a disc harrow was pulled and reduplication was made and the seed bed was made ready for planting. The planting activity was carried out by hand on 15.11.2018 by opening the drawings with a marker. Weed control in the experimental area was done with a hoe twice, before and after flowering. The harvest of the experiment was carried out on 12.06.2019. Measurement, counting and threshing processes of the harvested plants were made and their average values were taken. Parcel yields were calculated by threshing the plants after they were dried in bunches. Since the study was carried out in winter, irrigation was not done.

In the study, factorial trial design in random blocks was used to determine the differences between the doses of bacteria inoculation and vermicompost in terms of yield and yield components, and the Duncan (5%) Multiple Comparison Test (Düzgüneş et al., 1987) and Costat package were used to determine the different groups. programs were used.

#### RESULTS AND DISCUSSION

The average values of bacterial inoculation and vermicompost dose applications in terms of the plant features in the broad bean are given in Table 3.

Table 3. Averages of the Rhizobium inoculation and different vermicompost doses examined in the study

Plant features	Inoculation		Vermivompost Doses				
	Without	With	0	250	500	750	
			kg/da	kg/da	kg/da	ka/da	
Plant height (cm)	145.0 b	158.0	147.1	152.7	154.9 a	151.3 ab	
		a	a	a			
First pod height	47.6 b	62.2 a	42.6 c	54.05	60.6 ab	62.5 a	
(cm)				b			
Num. of	4.0 b	4.5 a	3.4 b	4.7 a	4.8 a	4.1 a	
branch(units/plant)							
Num. of pod per	17.6 b	23.6 a	15.9 c	22.0	23.5 a	21.1 b	
plant(units/plant)				ab			
Num. of seed per	64.7 b	96.3 a	54.1 c	84.6	99.5 a	84.0 b	
plant(units/plant)				b			
Grain yield (kg/da)	226.8 b	258.0	220.2	242.8	259.4 a	247.1 b	
		a	c	b			
Harvest index (%)	25.0 a	25.0 a	24.3 b	25.5 a	25.2 a	24.9 ab	
100 seed weight (g)	131.5 b	136.1	130.3	133.8	136.3 a	135.0 b	
		a	c	b			
Protein ratio (%)	23.5 b	24.6 a	23.4 b	24.2 a	24.4 a	24.2 a	
Number of nodules	41.0 b	99.7 a	49.8 c	65.1	83.1 a	83.3 a	
				b			
Weight of nodules	0.714 b	1.085	0.732	0.894	0.981 a	0.990 a	
		a	c	b			

It was seen that the data obtained from the rhizobium inoculation application were higher in all of the characteristics examined in the study, except for the harvest index. When the plant height was examined in terms of Rhizobium bacteria inoculation, the highest plant height was obtained from the inoculation application with 158.0 cm, and the lowest plant height was obtained from the application without inoculation (145.0 cm). The average plant height of vermicompost doses in broad beans varied between 147.1-154.9 cm. The highest plant height of 147.1 cm was obtained from the application of 500 kg/da fertilizer dose, and the lowest plant height of 147.1 cm was obtained from 0 kg/da (control) application. Saket et al. (2014) reported that vermicompost and compost fertilizers gave the highest growth parameters. Amin and Moghadasi (2015), in their study with different nitrogen doses (0, 90,120 kg ha<sup>-1</sup>) and vermicompost (0.15 tons ha<sup>-1</sup>) in Iran, the lowest plant height was found in the control plots, and the highest plant height was vermicompost reported their results from their practice. Tadesse et al. (2017) reported that both organic and inorganic sources increased yield and yield elements in their field study to investigate the effect of the integrated effect of vermicompost, rhizobium inoculation and NP fertilizers on the yield of broad bean. Chaichi et al. (2018) reported that vermicompost increased plant height in their study to determine the effect of vermicompost tea (VCT) as a natural foliar fertilizer on growth and reproduction. The results obtained by the researchers from their studies show similarities with the findings we obtained.

In the Rhizobium bacteria inoculation application, the highest value was found as 62.2 cm in the inoculated plots, and the lowest value was 47.6 cm in the non-inoculated plots. In terms of vermicompost application at the first pod height, the highest value was obtained from

the application of 62.5 cm and 750 kg/da fertilizer dose, and it was included in the same group with 500 kg/da fertilizer dose. The lowest value in fertilization applications was obtained from the control, that is, from the parcels that were not fertilized at all, with 42.6 cm. Generally, the first pod height values are also high in tall plants with large vegetative parts. Although the first pod height is a feature that is primarily affected by the genetic structure, environmental conditions also have a significant effect on the first pod height (Karaköy 2008). The height of the first pod is very important in terms of suitability for machine harvesting. On the other hand, studies on plants related to microbial, organic and inorganic fertilizer applications; Kaya et al., (2007), in their study examining the effect of organic (slempe) and commercial fertilizer, reported that they obtained the lowest first pod height from the control plots. Yeşilbaş (2015) reported that the lowest values in terms of first pod height from the organic and inorganic fertilizer application were obtained from the control plots, and the highest average values were obtained from the plots where chicken manure was applied. Bacteria inoculation increased the height of the first pod. Altınkaynak and Togay (2021) investigated the effect of organic and inorganic fertilizer applications and bacterial inoculation on yield characteristics of chickpea in Fethiye-Kayaköy conditions, and reported that bacterial inoculation increased the height of the first pod. The findings obtained as a result of the experiment confirm the findings of the researchers.

When the number of branches in the pod was examined in terms of Rhizobium bacteria inoculation, the highest number of branches was obtained from the inoculation application with 4.5, and the lowest number of branches was obtained from the application without the bacteria (4.0 units). It was determined that the average number of branches per pod of vermicompost application varied between 3.4 and 4.8. The highest value for the number of branches was obtained from the application of 500 kg/da vermicompost with 4.8 units, and the lowest number of branches was obtained from the 0 kg/da (control) application with 3.4 units. Yeşilbaş (2015) reported that the lowest value was obtained from the control plots with 2.4 and the highest value from the organic fertilizer application with 3.03 pieces/plant in the study in which he examined the effect of organic and inorganic fertilizers on the number of branches per plant in lentils in Van. Zeidan (2007), organic fertilizer application increases the number of branches, Saket et al. (2014) stated that the applied organic fertilizers (farm, chicken, compost and vermicompost) increased the number of branches, but the difference between them was not significant. Fufa and Amdemariam (2021), in their study in Ethiopia, investigated the effects of NPS and bio-organic fertilizers on yield and yield components in broad bean plants. At the end of the study, they reported that the farm manure Rhizobium application + 100 kg / ha NPS application gave the highest number of branches. The findings related to the number of branches obtained in the bacterial inoculation applied in the study conducted by Bildirici (2003) are partially similar to the findings obtained in this study and the results of the researchers.

When the effect of different inoculation applications on the number of pods per plant was examined, it was determined that the average values ranged between 17.6 and 23.6 units/plant. The average number of pods per plant of vermicompost doses ranged from 15.9 to 23.5 units/pod, and the highest value was obtained from 23.5 units/pod and 500 kg/da vermicompost dose and it was determined that it was statistically indifferent from 250 kg/da vermicompost dose. Karahan and Şehirali (1999) found that inoculation with bacterial culture and fertilizer application caused a significant increase in the number of pods in the plant compared to the control process, and as a result of the path analysis made by choosing the dependent variable of grain yield, the factors that directly and positively affect the grain yield were the number of pods per plant and the weight of 100 seeds they have reported. In studies on plants related to microbial, organic and inorganic fertilizer applications; Kaya et al., (2007), in their study investigating the effect of organic and commercial fertilizers, reported that the lowest number of pods in the plant was obtained from the control plots. Chaichi et al. (2018) stated that vermicompost tea in pod increased the number of pods in the plant. Fufa and Amdemariam (2021), in their study in Ethiopia, reported that the highest number of pods in the pod plant was obtained in farm manure + Rhizobium application + 100 kg/ha NPS application.

In terms of bacterial inoculation applications, the highest number of seeds in the plant was obtained from the inoculation with 96.3 units/plant, while the lowest number of seeds in the plant was obtained

from the uninoculated plots with 64.7 units/plant. The average dose of vermivompost varied between 54.1 - 99.5 pieces/plant. While the highest grain number value in the plant was obtained from 99.5 units/plant and 500 kg/da vermicompost dose, the lowest value was determined in the control with 54.1 units/plant. In studies on the number of grains in the plant; Genetic structure of the variety, environmental conditions and applied breeding techniques are effective in obtaining high yield per unit area. There is a positive and reliable relationship between the number of seeds and pods per plant and plant yield. Increasing the number of grains and pods in the plant also increases the grain yield in the plant (Güler et al., 2001). Kaya et al. (2007), in their study examining the effect of organic (slempe) and commercial fertilizers, reported that the lowest number of grains per plant was obtained from the control plots. It was reported that nitrogen fertilizer applications followed it and the highest values were obtained from vermicompost application. Amin and Moghadasi (2015) reported that the lowest values in the effect of vermicompost and nitrogen fertilizer applications on the number of grains in chickpea plant were obtained from the control plots, followed by the nitrogen fertilizer applications, and the highest values were obtained from the vermicompost application. Altınkaynak and Togay (2021) stated in his study that he investigated the effect of organic and inorganic fertilizer applications and bacterial inoculation on the yield characteristics of chickpea in Fethiye-Kayaköy conditions, and stated that bacterial inoculation increased the number of grains in the plant. The findings of the researchers and the findings obtained in this study support each other.

As seen in Table 4., the average grain yield per unit area in terms of grafting varied between 226.8-258.0 kg/da. In terms of fertilizers, the average grain yield per unit area in the pod varied between 220.2 -259.4 kg/da, and the highest unit area grain yield was determined from the vermicompost dose of 259.4 kg/da and 500 kg/da. The lowest unit area grain yield was obtained from the control with 220.2 kg/da. Bozoglu et al. (1997) found that inoculation of seeds with bacterial culture and fertilizer application (DAP) significantly increased grain yield in two years compared to control. Similar results were reported by Otienoe et al. (2007) have determined and reported that vermicompost increases grain yield per unit area. Amin and Moghadasi (2015) reported that they obtained the lowest grain yield from the control plots in their study where they applied vermicompost. Microbial, organic and inorganic fertilizer applications related to grain yield and studies on chickpea plant; Kaya et al. (2007), in their study on the effect of organic (slempe) and commercial fertilizers on chickpeas, showed that the lowest grain yield was obtained from control plots without fertilizer (108.8 kg/da), the results of commercial fertilizer and organic fertilizer (slempe) applications were close to each other. They reported that more grain yield was obtained from fertilizer application. Elkoca et al., (2008), in their study on microbial and chemical fertilizer applications, reported that there was a difference between applications, the lowest grain yield from control plots, and the highest grain yield from microbial fertilizer application. Pashaki et al. (2016) applied nitrogen fertilizer, phosphorus fertilizer, bio-fertilizer combination of Bacillus and Pseudomonas and two doses of vermicompost (0.7 tons/ha) to the

bean plant in their study in Iran. At the end of the study, they reported that the use of 100 kg nitrogen, bio-fertilizer and vermicompost per hectare and 100 kg phosphorus increased grain yield and yield components. Admasu et al.(2017) stated that both organic and inorganic sources increase yield and yield elements and should be used in both sources. Fufa and Amdemariam (2021), in their study examining the effects of NPS and bio-organic fertilizers on yield and yield components in broad bean plants, 4 NPS doses (0, 50, 100 and 150 kg/ha) and 4 different bioorganic fertilizers (commercial organic fertilizer, Rhizobium crossing, farm and farm manure manure+Rhizobium inoculation) and their combinations were applied. At the end of the study, they reported that the farm manure Rhizobium application + 100 kg/ha NPS application gave the most grain yield, while Birhanu (2021) reported that rhizobium bacteria inoculation (grafted and ungrafted), vermicompost doses (0, 3, 6 and 9 t/ha) and reported that inorganic fertilization (0, 60, and 120 kg/ha) significantly affected yield and yield components. The results obtained in this study are similar to the findings of the researchers.

As seen in Table 4., the average harvest index in terms of inoculation was found to be 25.0%. In terms of fertilizer doses, the average harvest index in broad bean varied between 24.3% and 25.5%, and the highest harvest index was determined between 25.5% and 250 kg/da vermicompost. The lowest harvest index was obtained from the control with 24.3%. Rudresh et al. (2005), the results obtained from their studies and the findings we obtained show similarities. Fufa and

Amdemariam (2021), in their study conducted in Ethiopia in 2016, examined the effect of NPS and bio-organic fertilizers on yield and yield components in broad bean plants, with 4 NPS doses (0, 50, 100 and 150 kg/ha) and 4 different bioorganic fertilizers (commercial organic fertilizer, Rhizobium inoculation, farm manure and farm manure+Rhizobium grafting) and their combinations were applied and they reported that at the end of the study, farm manure + Rhizobium application + 100 kg/ha NPS application gave the harvest index value. The results of the researcher and the results obtained in this study are partially similar.

In terms of bacterial inoculation, the highest 100 grain weight was 136.1 g without inoculation, and the lowest value was 131.5 g in the uninoculated application. While the highest 100 grain weight was obtained from 500 kg/da vermicompost application (136.3 g), the lowest 100 grain weight was obtained from the control application (130.3 g). Amin and Moghadasi (2015) reported that while the lowest hundred grain weights were obtained from the control plots in their study where vermicompost and nitrogen fertilizer applications were applied, the plots where commercial manure and vermicompost were applied were in the same group. Göksu (2012) reported that in Bursa, the lowest 100-grain weights were obtained from the control plots, while values close to control were found in bacterial applications compared to other applications, while the highest values were obtained from chicken manure applications. Kumar et al. (2014) stated that they obtained the highest 100 grain weight from the combination of chemical

fertilizer + 5 tons/ha vermicompost + Rhizobium + phosphate solvent bacteria in their study investigating the effects of vermicompost, biofertilizers and chemical fertilizer combinations in chickpea.

In Rhizobium bacteria inoculation, the highest protein rate in the seed was found in the inoculation application with 24.6%, while the lowest protein rate in the seed was found in the uninoculated application (23.5%). It was determined that different groups were formed between vermicompost doses in terms of protein content in the grain. The average protein content in grain obtained from different vermicompost doses varied between 23.4% and 24.4%. The highest protein content in grain was found with 24.4% from 500 kg/da vermicompost application. The lowest protein content was obtained from the control with 23.4%. Elkoca et al., (2008), in their study on microbial and chemical fertilizer applications, found that there was a difference between the applications, the average protein rate obtained in the first year was 24.8%, the average of the second year was 25.5%, and the lowest protein rate was 23.9% from the control plots. reported that the highest protein ratio was obtained from Rhizobium + nitrogen-fixing microbial fertilizer application (26.2%), while N and NP applications followed these values with a rate of 25.4%. Table 4, in pods in terms of Rhizobium bacteria inoculation. Saket et al. (2014) reported that the highest protein ratio was obtained from worm manure in their study using organic and inorganic fertilizer sources in chickpea. Alsina et al. (2016), in the study they carried out by applying four different Rhizobium strains to two broad bean cultivars in Latvia, they found that strain productivity

depends on the interaction between strain, soil conditions and cultivar, and protein contents vary according to cultivars. They reported that 407 promoted protein aggregation in the seeds of both cultivars.

When examined, the highest number of nodules was obtained from the inoculation application with 99.7 units/plant, while the lowest value was obtained from the uninoculated application (41.0 units/plant). The highest number of nodules was obtained from the application of 83.3 units/plant and 750 kg/da vermicompost dose and 500 kg/da application, which is in the same group, and the lowest value was obtained from 49.8 units/plant and 0 kg/ha (control) application. Otieno et al. (2007) are similar to the results obtained from the studies of researchers such as the ones we have obtained. Argaw and Mnalku (2017) investigated the efficacy of vermicompost and Rhizobium inoculation on nodulation, yield and yield characteristics of broad bean plant in Haramaya, east of Ethiopia, with five different doses of vermicompost (0, 2, 4, 6 and 8 tons ha-1). ) and two levels of Rhizobium inoculation (inoculated and uninoculated). Over the years, they reported that the highest average nodule number (298.00) was obtained from 4 tons ha of vermicompost application, with Rhizobium inoculation increasing the mean number of nodules and non-inoculated by 6% and 11%, respectively. Birhanu (2021), the effects of rhizobium bacteria inoculation (inoculated and uninoculated), vermicompost doses (0, 3, 6 and 9 t/ha) and inorganic fertilization (0, 60, and 120 kg/ha) on yield and yield components in broad beans. reported that nodulation was significantly affected by the applications at the end of the study they

investigated with the pot experiment. He reported that the highest number of nodules (101.8 units) was obtained in 6 t / ha application of vermicompost, and Rhizobium inoculation increased the average number of nodules by 35% compared to the uninoculated. Our findings support the results of the researchers.

Table 4, in terms of Rhizobium bacteria inoculation in broad bean. When examined, the highest nodule fresh weight was obtained from the inoculation application with 1.085 g, and the lowest value was obtained from the inoculated application (0.714 g). While the highest nodule fresh weight was 0.990 g and 500 kg/da vermicompost application, it was in the same group with 750 kg/da vermicompost application , the lowest value was obtained from 0 kg/da (control) application with 0.732 g. Argaw and Mnalku (2017) and Birhanu (2021) reported that both vermicompost and Rhizobium inoculation increased nodule weights in their studies in which they applied rhizobium bacteria inoculation (inoculated and uninoculated) and vermicompost doses in broad bean.

#### CONCLUSION AND RECOMMENDATIONS

This research was carried out in order to determine the effects of different bacteria inoculation and worm manure dose applications on the quality, environment, yield and yield components of pods in Muğla-Fethiye ecological conditions.

In this study, to determine the effect of bacterial inoculation and vermicompost dose applications, plant height, first pod height, number of branches per plant, number of pods per plant, number of pods per plant, grain yield per unit area, harvest index, hundred grain weight, protein ratio per grain, number of nodules and nodule fresh weight characteristics were examined.

While the average grain yield per unit area in terms of rhizobium bacteria inoculation varied between 226.8-258.0 kg/da, the highest unit area grain yield in terms of vermicompost applications was obtained from 259.4 kg/da and 500 kg/da vermicompost dose. The lowest unit area grain yield was obtained from the control with 220.2 kg/da.

Growth characters such as plant height, first pod height, number of branches per plant, number of pods per plant, number of grains per plant, grain yield per unit area, harvest index, protein ratio in grain and number of nodules were affected differently by different bacterial inoculation and vermicompost dose applications, and especially bacteria inoculation and vermicompost (500 kg/da) had a positive effect on these characters.

It can be said that very important results have been obtained from this study, which has been carried out today, where the demand for organic products has increased for reliable food supply, sustainable life and the environment. Considering the damage caused by the unconscious use of chemical fertilizers to the nutrient cycle and the increasing fertilizer prices, the organic-based fertilizers applied in this study gave positive results in terms of both environmental pollution and producing quality, healthy and cheaper food. In this context, it is recommended to grow the broad bean, which is one of the environmentally friendly legume

plants, with organic fertilizers, in order to evaluate our organic fertilizers correctly, to prevent environmental pollution, to add organic fertilizer to our soils and to obtain a very important and high quality protein source.

As a result of the study, it was determined that bacteria inoculation and vermicompost applications in the region provided significant increases in the characters closely related to quality, environment, yield and yield elements in the broad bean.

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## **CHAPTER 8**

## THE IMPORTANCE OF EDIBLE GRAIN LEGUMES IN ORGANIC AGRICULTURE

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#### INTRODUCTION

The rapid rise of the world population increases the importance of agriculture in human life. Thus, in the face of increasing food demand, producers use various methods to obtain more products per unit area. However, the fact that the agricultural inputs they use intensively deteriorate the soil structure, causing various problems such as salinity problems and environmental pollution adversely affecting human health. Pesticides, fertilizers, etc. are used in crop production. While chemicals provide an increase in efficiency, their excessive use is increasingly damaging to the environment (Akman and Kara, 2001). Over the years, continuous monoculture agriculture has caused the excessive proliferation of pests and diseases as a result of not choosing crop rotation, while the excessive and unconscious use of synthetic chemical pesticides used to combat them has led to the disappearance of beneficial breeds and has led to the deterioration of the biological balance. The use of more synthetic mineral fertilizers to get more products from the unit area, these fertilizers, which had used more than necessary, have polluted the groundwater and created nitrate pollution to a level that harms animal and human health. In agriculture, the health and quality of the product have been left to the second plan. In addition, as a result of unconsciously increasing mechanization, both the living layer of the soil was destroyed, and the risk of erosion, increased as a result of the formation of solid layers (Altındişli et al., 2000; Ölgen et al., 2009).

Sustainable agriculture is the creation of an agricultural structure in which agricultural technologies that do not harm the environment and living things are used together with the protection of natural resources in the long term. While there are many applications such as the protection of water and soil resources and integrated drug management in sustainable agriculture, organic farming techniques that meet quality, health, and environmental standards by avoiding the use of unnatural inputs such as pesticides and synthetic fertilizers play an important role (Turhan, 2005).

With the spread of intensive agriculture in the world, the increase in the use of pesticides and chemical fertilizers, the use of additives, and the inclusion of genetically modified products in the production cycle, the amount of agricultural production has increased, and accordingly, the threats to the natural balance and the health of living things have also risen. For this reason, the importance of organic agriculture has increased in recent years and has gained a commercial dimension as a farming type (Yeşilbağ, 2005).

Organic farming has been reported to be used as organic in England, ecological (ökülogisch) in Germany, and biological (biologique) in France due to language differences (Demiryürek, 2004). Organic fertilizers applied to the soil in organic agriculture improve the water holding capacity of the ground, aeration, and nutrient availability in the soil (Jackson et al., 2003). Organic farming systems produce lower yields compared with conventional agriculture. However, they are more profitable and environmentally friendly and deliver equally or

more nutritious foods that contain less (or no) pesticide residues, compared with conventional farming (Reganold and Wachter, 2016).

#### **EDIBLE GRAIN LEGUMES**

The Leguminosae (Fabaceae) family is the third-largest family with 750 genera and 20,000 species (Hulse, 1991). Edible legumes are important plants for human and animal nutrition, improving the physical, chemical, and biological structure of the soil, and economically, in the Fabaceae family Fabales order (Sepetoğlu, 2002). In order for people to continue their lives, they need a balanced and adequate diet. Protein is of great importance in meeting this nutritional need. 70% of the world's protein needs are supplied from plant sources. Vegetable proteins consist of 66% cereals, 18.5% edible legumes, and 15.5% other plant sources (Hasancaoğlu, 2016). Edible legumes, which are very effective on nutrition, are divided into two groups as cool and warm climate legumes. Cool climate legumes include broad beans (Vicia faba L.), chickpeas (Cicer arietinum L.), lentils (Lens culinaris Medik.), and peas (Pisum sativum L.), while hot climate legumes include cowpeas (Vigna ungiuculata L.) and beans (Phaseolus vulgaris L.) (Karagül, 2017).

Edible legumes, which are a source of vegetable protein, are very significant in terms of being rich in vitamins and minerals. In addition, they are effective in reducing the risk of cardiovascular diseases, obesity, and cancer with their antioxidant content. In addition, they are suitable products for high blood pressure and diabetic patients (Pekşen

and Artık, 2005). As a protein source, legumes have an important place in the diet of people living in underdeveloped and developing countries. In this respect, the consumption of legumes per capita, which was 12 kg in the 1960s, has decreased to 7.5 kg/person today. However, today, in many countries (India, Brazil, etc.), legumes are still consumed at a very high level (10-20 kg/person) (Sehirali et al., 2000).

The fact that they cost less than animal proteins and can be stored for longer periods without spoiling increases the importance of legumes (Erdin and Kulaz, 2014). Small grains and straw of legumes, whose dry grains are consumed, are used as a protein source in animal nutrition. Among the edible legumes, chickpeas, lentils, broad beans, peas can be grown both in summer and winter, while beans and cowpeas can only be grown in summer (Uçar, 2021). Edible grain legumes are the most produced field crops after cereals. In terms of production area and production amounts, while 77,402,249 tons of production is made on an area of 75,776,593 ha in the world in 2019, the production amount in Turkey is 1,217,700 tons in 2019 and 1,288,197 tons in 2020 (FAO, 2021; TUIK, 2021). Chickpea cultivation is carried out in almost all regions. Red lentils are grown in Southeastern Anatolia and green lentils are mostly grown in Central Anatolia (TUIK, 2021).

Edible grain legumes, which are rich in nutrient content, have regulated and healing properties in soil structure. Rhizobium bacteria living in the same environment with legumes increase the amount of organic nitrogen in the soil by binding the free nitrogen in the atmosphere. The nitrogen fixed by this method varies between 5-20 kg/da annually (Şehirali, 1988). Due to these features, it is producer-friendly, increases the level of organic matter in the soil, increases aeration and water holding capacity, and ensures that the products grown are healthier, higher quality, and nutritious (Adak et al., 2015). For these reasons, it is very important to grow edible legumes in crop rotation.

The production of edible legumes in small-scale agriculture causes low yield and profitability rates and high-cost rates. In the face of these situations, the fragmentation of agricultural lands should be prevented and the efficiency and quality parameters should be increased by applying a suitable production model by making consolidation (Ton et al., 2014).

# THE PLACE OF EDIBLE GRAIN LEGUMES IN ORGANIC AGRICULTURE

Organic agriculture is a production model that aims to protect the soil by prohibiting the use of chemical fertilizers and pesticides, preferring organic and green manure, and adopting alternation, plant resistance, and biological control methods as a principle, ignoring yield and increasing quality (EBSP, 2018). The procedures on organic farming are determined by the regulation. According to the Regulation on the Principles and Implementation of Organic Agriculture, soil fertility, sustainability, protection and development of biodiversity, prevention

of soil erosion and compaction, and nutrition of plants through soil ecosystem are essential in organic agriculture (Anonymous, 2010). According to this article, tillage cannot be carried out in a way that will disrupt the physical, biological, and chemical structure of the soil. In the overturned soils, the particles carried down by the precipitation remain stuck on the plow base due to the pressure of the tillage and similar tools entering the field. The layer, which becomes stronger, harder, and more prominent over time, can limit the active root depth of the plants. The hard layer, which is formed naturally or with the effect of processing tools, also reduces the aeration and infiltration capacity of the soil profile (Eser et al., 1998).

Edible legumes provide aeration of the soil by opening deep channels in the ground with their pile roots. It has been reported that reduced tillage methods are used in legume-cereal rotation, weeds and pathogens are reduced, and the need for nitrogen fertilizers is reduced (Nemecek et al., 2008).

According to the Regulation on the Principles and Implementation of Organic Agriculture, legumes and deep-rooted plants are grown or green fertilization is made within the perennial crop rotation program. It is allowed to use animal manure or organic materials from organic production, preferably both composted. In order to agricultural nitrogen from causing water pollution, the total amount of animal manure to be used in organic plant production cannot exceed 170 kg/N/ha/year. This limit is only; It is applied in the use of composted farm manure and liquid animal manure, including farm

manure, dried farm manure, dried poultry manure, composted animal manure, poultry manure (Anonymous, 2010).

Crop rotation and green manuring, which are of great importance for the realization of sustainable agricultural systems, significantly affect the performance of agricultural production. Crop rotation also has a great effect on soil fertility (Schönhart et al., 2011). Organic crop producers, especially in developed countries, are in great demand for crop rotation and compost applications in order to increase soil fertility (Delate et al., 2003). The primary and most important benefit of green manure, which helps to get more and quality products by increasing the productivity of the soil, is to enrich the soil in terms of organic matter. As green manure plants; legumes, cereals, and other families (mustard, rapeseed, radish, poppy, safflower, turnip) plants are used alone or as a mixture (Karakurt, 2009). It is recommended that plants that will be rotated and used for green manure should be selected among legumes (Dhavan et al., 1991; Duman and Algan, 2012; Aslan et al., 2013). The plant residues remaining after the harvest of edible legumes are broken down in the soil, increasing the organic matter content of the soil, contributing to the increase of permeability, and facilitating the uptake of other nutrients (Crozat and Fustec, 2004). It has been reported that the yield of cereals grown after legumes increased (Uzun et al., 2005).

The use of biodynamic preparations is allowed. Nitrogen fertilizers obtained by chemical methods cannot be used. If sufficient soil

fertility and biological activity cannot be achieved in organic plant production areas, suitable non-genetically modified plant-based preparations or microorganism preparations are used for compost activation. In order to increase the usefulness of soil conditions and nutrients in the soil or in the plant, non-genetically modified microorganism preparations that are generally allowed to be used in agricultural production in our country are used (Anonymous, 2010).

According to the Regulation on the Principles and Implementation of Organic Agriculture, non-genetically modified microorganisms are used as microbial fertilizers. Symbiotic, asymbiotic bacterias and mycorrhizae are used for this purpose. Leguminous plants are significant in converting the free elemental nitrogen in the air into a form that can be used by plants through Rhizobium bacteria. Edible legumes meet the nitrogen they need through these bacteria by establishing a symbiotic relationship with Rhizobium bacteria, which are in the same cross-grafting group living in the root zone. 12.000 leguminous species are hosts for Rhizobium bacteria, and considering that 200 of them are grown, the potential of nitrogen fixation and the importance of legumes emerge (Meyveci et al., 2005). In this respect, they contribute to meeting the nutritional element needs of the products to be grown after legumes. In this way, nitrogen gain is between 5-19 kg N/da per year under suitable conditions. These values are 19 kg/da in broad beans, 12 kg/da in lentils, 9 kg/da in peas and cowpea, 8 kg/da in chickpeas, and 5 kg/da in beans (Geçit, 1995; Soysal and Erman, 2020).

## **CONCLUSION**

Applications to be made in organic agriculture are determined according to the Regulation on the Principles and Implementation of Organic Agriculture. According to this regulation, the use of genetically modified materials, the use of chemical fertilizers and pesticides, and the cultivation of the soil in a way that disrupts the structure of the soil are prohibited. With the inclusion of legumes in the production cycle in organic farming, the soil is less processed and the physical, chemical, and biological structure of the soil improve. In addition, some of the fertilizer needs are met with symbiotic nitrogen fixation. Aeration and permeability of the soil are increased by the pile roots of legumes. Because of these aforementioned characteristics, the inclusion of legumes in the organic farming system is important both economically and ecologically.

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#### **CHAPTER 9**

# USAGE AREAS OF HAYIT (Vitex agnus-castus L.: VERBENACEAE) PLANT AND ITS IMPORTANCE IN ORGANIC FARMING

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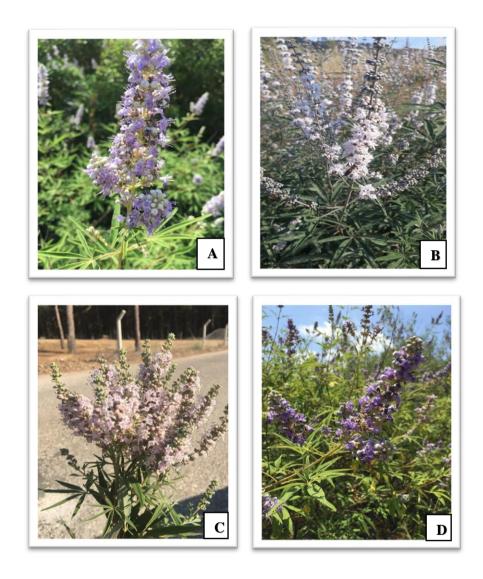
#### **INTRODUCTION**

The use of plants for various purposes is based on ancient times. It is known that they have been used for food, spice, dyestuff, medicine and treatment since ancient times, and it is seen that they are also still used for different purposes today. Although synthetic and semi-synthetic drugs have taken precedence over herbal medicines with the advancement of technology and science, interest in natural substances (plant essential oils and extracts) and medicinal and aromatic plants has increased in recent years, especially in developed countries (Ma, 2010). The interest in herbal and natural products is adopted by today people with the belief that these products are safer, low-cost, easily accessible and have fewer side effects (Ababutain & Alghamdi, 2018). It is estimated that there are over 10,000 of these herbal products such as the plant essential oils and their extracts in the world. These products, which have been produced and consumed since prehistoric times, prove the words of Hippocrates, "make your food your medicine, your medicine your food" (Varlı et al., 2020). The demand for organic products has increased as a result of the increasing importance given by consumers to protecting their own health and the environment, especially in developed countries. The demand for organic products not grown in developed countries has led to the development of international trade. Thus, developing countries such as Turkey, whose ecology is suitable for organic agriculture, become organic product producers and exporters in order to meet the demands from developed countries (Demiryürek, 2011). Organic farming is a production system that maintains soil, ecosystem and human health. The system, instead of using inputs with negative effects; ecological processes are based on biodiversity and cycles adapted to local conditions. Its rich biodiversity, clean ecological areas, plant varieties resistant to diseases and pests, and low level of chemical input use are among the main advantages for the development of organic farming in our country as well as in developing countries. Therefore, Turkey has a great potential to grow many products with its ecology, geographical location and topographic structure. Production areas and product diversity in the medicinal and aromatic plants sector in Turkey are increasing day by day. The main reason for this is the increase in the demand for the products obtained from these plants in the world. Production systems that evaluate many different points such as inputs used in production, registration system, environmental effects and traceability have gained importance recently. Organic farming practices are production models that contain these parameters and are monitored and certified by authorized certification bodies with laws and regulations (Karik, 2015). There are more than 9500 plant species in the flora of Turkey, of which 3400 are endemic (Davis, 1965-1988; Güner et al., 2000). Brassicaceae, Lamiaceae, Leguminosae, Apiaceae and Asteraceae families, which have many important plants, are involved in scientific studies, imports and exports (Uygur et al., 1990; Özdemir, 2007; Büyükkurt et al., 2016). The Lamiaceae family, which includes a significant part of medicinal and aromatic plants, is the first among the plant species that are imported and exported today. The family includes 250 genera and 7000 species worldwide (Ulcay & Şenel, 2018; Kahraman & Doğan, 2010). In Turkey, there are 844 species, 326 of which are endemic, belonging to

this family (Anonymous, 2020a). The family includes medicinal and aromatic plants such as sage (Salvia spp.), thyme (Origanum spp.), mint (Mentha spp.), basil (Ocimum spp.) and lavender (Lavandula spp.) (Aktas, 2001; Bagei & Koçak, 2008; Erdogan, 2014; Karik, 2015). These plants, which are rich in essential oils, have been used in many studies. Essential oils are usually colorless or light yellow, volatile, strong-smelling, natural products that are obtained from the leaves, fruit, bark or root parts of plants, are liquid at room temperature, and can sometimes crystallize (Ceylan 1983; Kılıç 2008). Essential oils prevent the growth of some yeasts and bacteria, and due to these properties, they are the natural preservatives of food. In Turkey, a total of 1010 tons of thyme, sage and mint in 2017 and a total of 293 tons of products in 2018 were certified for organic production (Anonymous, 2020b). Medicinal plant *V. agnus-castus* L. belonging to the *Vitex* genus of the Verbenaceae family. It is represented by two species (Vitex agnus-castus L. and Vitex pseudo negundo Haussknecht) in the flora of Turkey (Ervigit et al., 2015). V. agnus-castus plant, whose homeland is the Mediterranean countries, also grows in Western Asia and East Africa (Brickell & Zuk, 1996; Blamey & Grey-Wilson, 1998). In our country, it is distributed mainly in Muğla, Antalya, Adana, Mersin, İskenderun, Bursa, Trabzon, Giresun, İzmir, Manisa, Aydın, as well as Eastern Black Sea, Marmara, Aegean, Mediterranean, and partially South Eastern Anatolia regions (Kayacik, 1966; Anonymous, 1991). Hayıt plant (Vitex agnus-castus L.), which is among the medicinal and aromatic plants, is also called "Hayıt", "Ayıt", "Acı Ayıt", "Ayıd", "Priest's pepper", "Chaste tree", "Feminine grass" or "Five finger

grass" among the Anatolian people and is used in different ways (Baytop, 1963). Also, it is known as "Chaste tree", "Chasteberry", "Hemp tree", "Monk's pepper" in the UK; "Möchspfeffer", "Keuschlamn" in Germany, "Agneau-chaste" in France and "Vitice", "Agnucasto", "Lagano" in Italy (Özkan, 2010; Kuruüzüm, 2008). The word hagnos (chaste) was used for the plant in ancient Greece. However, it was confused with the Latin word agnus (lamb = symbol of purity in Christianity) in Christians. Agnus-castus means "pure, clean lamb" in Latin (Baytop, 1995). Due to the use of elastic branches in the construction of knitted baskets and fences, it is thought that the genus name comes from the Latin word vitilis (made by knitting) (Kessel, 2004).

Vitex agnus-castus L. (Hayıt) plant is in bush or shrub form, 3-6 m high, with a rounded crown, deciduous in winter, flowering in June-July, cluster-shaped white, pale pink, lilac, blue or purple bells. It is a species that blooms in the form of flowers (Figure 1). Its leaves are opposite, 3-7 segmented, long, oval and pointed towards the tip (Baytop 1963; Öztürk et al., 1990; Tanker et al., 1993). Its spherical seeds, which are bitter, special smelling and hard, are 3-4 mm in diameter and grayish brown in color (Davis, 1965-1988; Brickell & Zuk, 1996; Cheifetz et al., 1999) (Figure 2). The general growing conditions of Hayıt are stream beds and rocky areas, but it has been determined that some species that are resistant to salinity are seen in sandy areas near the seaside (Brickell & Zuk, 1996).



**Figure 1**: **A**-Lilac, **B**-White, **C**-Pale pink, **D**-Purple colored flower structure of *Vitex agnus-castus* L. (Hayıt) (Usanmaz Bozhüyük. 2021)

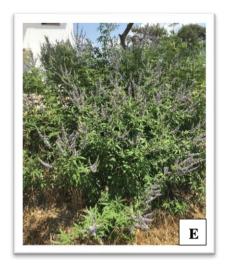
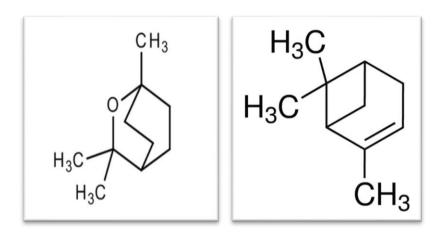




Figure 2: General view (E) and seeds (F) of Vitex agnus-castus L. (Hayıt) (Usanmaz Bozhüyük. 2021)

The essential oil obtained from the flowers of the Hayıt plant, which is rich in essential oil, is used instead of thyme oil in Muğla and Aydın provinces (Dülger et al., 2002; Ugurlu & Secmen, 2008). The essential oil ratios and components in the plant differ according to the organs of the plant, the development period, climate, genetics, environment, age and topographic conditions (Ceylan, 1995). According to the literature records; It has been determined that the main components that enable the emergence of essential oil character of V. agnus-castus plant during both flowering and fruit ripening periods are 1,8-cineol and α-pinene (Figure 3.) (Fakir et al., 2014; Varcin & Kesdek, 2020). In general, in different parts of the world; In studies on the essential oil content analysis of *V. agnus-castus* plant, it was determined that although the component ratios in the oil content changed, the components showed a similar distribution (Kustrak et al., 1994; Zoghbi et al., 1999; Sørensen & Katsiotis, 2000; Senatore et al., 2003). In addition, the rich content of essential oil contains bicyclic diterpenes (labdane and klerodan type), diterpenoid alkaloids, iridoid glycosides, flavonoids and triglycerides, which are mainly secondary metabolites (ESCOP, 2003).



1,8-sineol α-pinen **Figure 3**. Main components of *Vitex agnus-castus* L. (Hayıt) essential oil. (www.tcichemicals.com, www.sigmaaldrich)

The usage areas of the *V. agnus-castus* plant, which is preferred for various purposes, are given under the following headings:

# Use of Hayıt as a Medicinal Plant

The use of Hayıt, a medicinal plant, dates back to 2500 years ago. It is seen that even in the Hittites period, the plant was used as a medicine together with its seeds and it was transmitted orally and in writing until today (Ertem, 1974). B.C. In 450, Hippocrates stated that herbal leaf pulp was used in the treatment of areas of inflammation and swelling, and that it had a wound-healing effect, and said, "When there is any problem in menstruation, make the woman drink brown wine infused

with chasteberry". In the 1<sup>st</sup> century AD, roman physician Dioscorides, in his work "Materia Medica", recommended herbal porridge against headache and mentioned its use in menstrual disorders, and also stated that it increased milk secretion. Theophrastus and Plinius also mentioned the use of the plant in gynecological diseases (Özkan, 2010; Hobbs, 1996). The V. agnus-castus plant seeds, which was called the "tree of honor" in Ancient Greece, the "symbol of honor" in Homer's Iliad, and the "women herb" among the people, are used to treat gynecological diseases, menstrual irregularities and it is known that used as a regulator of spleen and liver activities in the treatment of infertility (Asker et al., 2006). Hayıt was preferred by monks as a spice in meals to suppress sexual desires in the Middle Ages. Because of this feature, it is called "Monk or Priest pepper" (Hobbs, 1996). It was also stated that Hayıt was effective against poisonous insect and snake bites in ancient times (Özkan, 2010). It was also used as a calming herb in nervous disorders in the Islamic period (Hoberg et al., 2001). Islamic scholars El-Kindi and El-Samarkandi stated that they use chasteberry along with other herbs for epilepsy and mental illnesses. Today, chaste seeds are sold in Egyptian markets against nervous disorders. Leung & Foster (1996) explained that John Gerard recommended chasteberry for uterine inflammation and reported that the infusion obtained from its seeds could be used in liver and spleen diseases. The widespread use of the herb in Europe began to decline since the beginning of the 1700's, and Cazin, the well-known medicinal herbalist of this period, introduced Hayıt as the plant that "takes the excitement of Venus, the goddess of love" (Özkan, 2010). In 1930, in Germany, Gerhard Madaus tested which part of the chasteberry plant was most effective in gynecological diseases with experimental animals and determined that fruit extracts were more beneficial. Later, in 1938, Madaus took the patent and developed a drug called "Agnolyte" and this drug was used in many scientific researches (Hobbs, 1996; Hobbs & Blumental, 1999). In Europe, extracts of the chasteberry plant were used in phytotherapy in gynecological diseases, infertility and premenopausal hot flashes for treatment purposes in the 1940-1950s (Mills, 1985; Hobbs, 1996; Blumental, 2000). Today, most of the women prefer capsules consisting of drugs or extracts prepared from the herb against the side effects of drugs (Schellenberg, 2001).

The using areas of the chasteberry plant vary from region to region. For example; it has been determined that a tea glass of infusion prepared by brewing the fruits and leaves of the plant in boiled water is good for intestinal and stomach disorders, abdominal pain and diarrhea in Muğla, Aydın and Çanakkale regions (Honda et al., 1996; Bulut & Tuzlacı, 2015). Also, in these regions, against fungal diseases on the hands and feet, the diseased area is dipped into hayıt, which is boiled in water, or steamed. In gynecological diseases, the patient is placed in the boiled steam warm water of the hayıt plant (Dülger et al., 2002). Again, it is known that porridge prepared from chasteberry leaves and fruits is applied to children with stomachache in Buldan, Denizli. In Urfa province (exam.: Siverek), it is reported that the infusion prepared from chasteberry seeds has a preventive effect on premature births (Tumen & Sekendiz, 1989; Baytop, 1999).

Another form of treatment using the leaves of the havit is rheumatic pains. In general, three application methods have been identified. When the leaves are fresh, they are wrapped directly on the painful area or the leaves are crushed or the leaves are boiled and applied as a hot compress in the form of porridge. Another application; it is the treatment of fresh, young leaves by crushing and boiling, adding flour and olive oil, wrapping the painful area, waiting for 7-8 hours. This and similar applications were applied in ancient times. Dioscorides used the leaves of the chaste plant in wounds and dislocated joint pain, while Plinius mixed the leaves with potassium nitrate, salt and wax in sprains, and suggested the use of seeds and leaves as a moxibustion in tendon diseases (Baytop, 1999). These application forms from the past to the present have been seen in different regions. For example, havit porridge is applied to rheumatic diseases in Burdur and Denizli (Buldan) provinces. The chaste leaves are burned with tarhana, vinegar and onions and applied to the aching area (Ertuğ et al., 2004; Özçelik & Balabanli, 2005). It has been observed that the chasteberry plant is also effective in diabetes and cardiovascular diseases. Although the application forms vary, in general, the leaves and seeds are brewed and drunk on an empty stomach. In addition, the ground seeds are used as a spice by sprinkling them on the dishes (Arituluk & Ezer, 2012).

# Use of Hayıt as Ornamental Plant in Landscape Areas

Vitex agnus-castus L. (Hayıt) plant, which is evaluated as wet and dry, is also used in landscape areas as an ornamental plant when evaluated visually. It is among the plants preferred in vegetative landscape areas with its spike-shaped, long and plump flower structure, its color range from white to purple, its long-lasting flowering without fading, its large seeds, and its tree-like shape and being suitable for pruning (Dehgan, 1998; Cheifetz et al., 1999; Burnie, 2000). At the same time, low-income people in rural areas use the branches, stems, roots and stumps of the chaste tree as fuel.

## Use of Hayıt as Basket, Saddle, Sling and Slingshot

In addition to its importance in medicine, pharmacy, medicine and landscaping, it has been determined that the chasteberry plant is also used in making baskets, saddles and pans in Anatolia. Since the branches of the chaste tree are flexible, hard and durable, they are especially preferred in basket making. Again, by making use of the flexibility of chaste branches, it has been determined that children use it in making slingshots for bird hunting and that the local people prefer to tie broomsticks (Kolancı, 2017).

# Use of Hayıt as Herbal Paint

Another area where chasteberry is used is as a vegetable dye or organic dye. Linen cloths, woolen fabrics and various textile products are dyed yellow with the water extracted after the chaste branches are boiled (Dülger et al., 2002).

# **Use of Hayıt in Honey Production**

The chasteberry is an attractive species for honey bees and its flowers are a good source of nectar. In the southern part of the Aegean Region, "Hayıt honey" is a type of honey that is consumed with fondness.

Especially in Aydın and its surrounding provinces, an average of 100-120 tons of chasteberry honey is produced annually (Uçak et al., 2017).

## **Use of Havit Against Insects**

In order to get rid of lice, fleas and other insects 15-20 years ago, local women used the branches of the chasteberry plant for washing clothes. Again, chaste branches were placed between the bed and the guilt in order to prevent moths (Kolanci, 2017). In some regions of Anatolia, the powder obtained from the seeds and leaves of Hayıt has been used to prevent mothballing of woolen fabrics (Baytop, 1984; Tümen & Sekendiz, 1989).

## **Use of Essential Oils and Extracts of Havit Plant**

It has been mentioned that the flowers and seeds of the chasteberry plant were used in perfume in the past years (Kolanci, 2017). Today, it is known that essential oils or plant extracts are used in perfumes, creams, soaps and many other cosmetic products. Again, in laboratory studies, it is seen that the oil and extracts of the chasteberry plant have been investigated against many gynecological parameters using mice as subjects (Haerifar et al., 2020; Hameed et al., 2021). At the same time, with the phytochemical and antioxidant studies, in recent years, especially the essential oil obtained from the seed and flower part of the chasteberry plant and the extracts obtained from different solvents, the antibacterial, antifungal and insecticidal effects have been emphasized (Balpinar et al., 2019; Bakr et al., 2020; Varcin & Kesdek, 2020; Zazharskyi et al., 2020). For example, in one of these studies, the essential oil of the hayıt plant used as larvicide against pine processionary month (*Thaumetopoea pityocampa* (Den. & Schiff., 1775) (Lepidoptera: Notodontidae)) and it caused the mortalities up to 100% (Varcin & Kesdek, 2020). In another study, Kordali et al. (2008) determined antifungal, phytotoxic and insecticidal effects of essential oil of *Origanum acutidens* L. and its three components, carvacrol, thymol and p-cymene.

When this and similar studies are reflected on the field, healthy, safe and chemical-free products will be produced, especially in organic farming, which is an environmentally friendly production system. In this way, human and environmental health will be significantly protected and will further increase the importance of organic farming.

### **CONCLUSIONS AND SUGGESTIONS**

In this study, the general characteristics and usage areas of *Vitex agnus-castus* L. (Hayıt) plant are mentioned. It has been seen that this plant, which is used in many fields and has a rich content, cannot be evaluated enough today compared to the past periods. Rapid urbanization and industrialization, rapid disappearance of vegetation in urban and rural areas, people's distance from being intertwined with nature, intense work tempo, stress and time constraints are the main reasons. It is a fact that the demand for natural products has increased due to the Covid-19 pandemic and the tendency for organic products is more in this troubled period. In addition, because the chemicals used in convantional farming leave residue on the products and cause various diseases such as cancer in humans, today's people have given more importance to organic

farming and consuming organic products obtained from organic farming. However, scientific studies have been carried out against viruses and medicinal plants in many areas (Asif et al., 2020; Salem et al., 2021). Especially thyme, mint, sage, lavender, ginger, turmeric etc. plant utilization rates have increased. While some of the plants in question are collected from nature, some of them are cultivated and produced. It should be ensured that the chasteberry or honey is included in these plants to increase the usage areas, to cultivate organic farming and to focus on more scientific studies. In the study, some suggestions can be made with the plant V. agnus-castus (Hayıt) based on the information and evaluations based on the literature.

- In our country and in the world, although havit flowers, leaves and seeds are used in the field of health such as stomach and stomach ailments, diabetes, cardiovascular diseases, rheumatism and joint pains, and especially gynecological diseases and disorders, microbial studies especially on essential oils and extracts are emphasized. Scientific research on its use as a medicinal drug should be increased.
- The importance of "Hayıt honey", which is little known in the food and agriculture sector, should be emphasized and its production and use should be encouraged, and it should be brought to the market as an organic product.
- It should be emphasized that it is one of the plants that can be used for coloring organic products in the textile industry.

- It should be evaluated in landscape areas with its cluster-like and colorful flowering structure and its feature of shrub form, and studies should be carried out to show that it can be used as a soil-retaining plant in eroded areas.
- By bringing the production of baskets, saddles and panniers, the use of which has decreased today, to the market, our natural wealth should be evaluated and as an alternative to reducing the pollution of bags and plastic, recyclable and durable shopping, market bags and different products should be designed with chaste branches.
- In the organic farming production process, extracts or essential oils of some medicinal-aromatic plants are among the preparations allowed to be used in combating diseases and pests. It has been determined that successful results have been obtained in the fight against diseases in both plant and animal production. By evaluating the essential oil and extracts of the chasteberry plant, natural sourced products should be developed instead of agricultural chemicals, and care should be taken to ensure that they are easy to access, cheap and practical.

In this study, *Vitex agnus-castus* L. (Hayıt), one of nature's rich plants, has been discussed in order to attract the attention of many sectors and researchers, to increase awareness, to evaluate it in different work areas, to shed light on future studies and to create ideas.

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## **CHAPTER 10**

# UTILIZATION OF SAGE (Salvia officinalis L.) AND THYME (Thymus Vulgaris L.) AND THEIR PLACE IN ORGANIC AGRICULTURE

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#### INTRODUCTION

Organic farming, a model of production for the re-establishment of the disrupted balance of nature, has grown in importance as a result of the recent use of chemicals such as fertilizers and pesticides in conventional agriculture. This production model is the agricultural system implemented with a nature-responsible approach without the use of chemical fertilizers and agrochemicals, protecting plant feeding and healthy soil management. In this context, the use of plant residues and extracts against plant diseases and economically damaging pests is important in biological control. Medicinal and aromatic plants have antifungal, antipathogenic, antimicrobial, herbicide, antiparasitic, antiprotozoal, antioxidant, anti-inflammatory, insecticide, and acaricide properties due to the compounds they contain. So in organic farming, these plants can be used as alternatives to chemical fertilizers and pesticides. Also, the best way to achieve healthily and quality products in organic farming is to produce close to natural conditions. Therefore, the cultivation of medical and aromatic plants only in organic farming is important.

Sage "Salvia officinalis" and thyme "Thymus vulgaris", commonly used as spices since ancient times, are important medical and aromatic plants belonging to the Lamiaceae family. Perennial, herbaceous, evergreen sage, and thyme have been widely consumed in the Mediterranean for various purposes, such as complementary and traditional medicine for thousands of years. In the past, it was used in Greek temples and

funerals, religious rituals as a fragrance and insecticide, and in Egypt as antimicrobial and preservative.

'Why does a person who grows sage in his garden die?', which was said by the medieval Salernitam Medical School in ancient Italy, is a phrase that shows its importance for health. Due to sage's active ingredients including "camfor", "carnosol" and "1, 8-cineole" (Jakovljević et al.,2021), especially rosmarinic acid (Santos et al., 2020), which has positive effects on blood sugar control, it shows antioxidant (Jakovljević et al., 2021), anti-inflammatory, antibacterial (Mendes et al., 2020), antifungal (Ivanov et al., 2021) and antiviral effect (Okaiyeto et al., 2021). Excessive consumption of sage also causes brain (Pelkonen et al., 2013), heart, and kidney damage, because it contains a compound called 'thujone' (Xie et al., 2019).

Thyme, known as the plant of nobility and courage in the ancient Greek period, is one of the plants with the highest virucidal and bactericidal action. Due to the active substances "carvacrol" and "thymol" contained in thyme, it has an antibiotic, antibacterial, antifungal, and antiviral effect (Mondal et al., 2021). Thyme oil is used in folk medicine against rheumatism, stomach, head, and muscle pains. Again, it is known to be used to lower cholesterol and blood sugar and stabilize blood pressure, as well as for respiratory diseases such as cough and bronchitis. Thyme oil and juice are considered beneficial for dental and gum diseases, insect bites, and eczema, as well as used in perfumery and employed as natural preservatives for hair, skincare.

# 1. THE USE OF SAGE AND THYME DURING THE PANDEMIC PERIOD

Diseases such as MERS, Ebola, Zika, and COVID-19 that occur in humans due to drought and water scarcity or climate change effects such as floods lead to very serious health problems. Medical and aromatic plants can be used against the coronavirus, which is spreading around the world today and is causing the most important pandemic. In research related to this issue, it has been stated that the use of traditional spices in our daily meals can help fight COVID-19 and that SARS-CoV-2 is better inhibited due to the Luteolin contained in sage (Kumar et al., 2020). Sage from commonly found medicinal plants should be considered for symptomatic management of COVID-19, viral and bacterial infections, many diseases including the heart and digestive system (Boone et al., 2020). It has been reported that sage has an antiviral effect and therapeutic properties against SARS-CoV-2 and that this inexplicable and widely available plant can be used in low-income regions, where it produces strong antiviral activity against SARS (Le-Trilling et al., 2020). It has been revealed that sage and thyme have an antiviral effect against IBV infection, which causes damage to the kidneys caused by COVID-19 (Lelešius et al., 2019). In a study examining the antiviral properties of aromatics, it was reported that sage and thyme have a bioactivity effect against DNA/RNA viruses, so they can be used in antiCOVID-19 studies (Tshibangu et al., 2020), and in clinical trials on these plants, it was reported that these plants can be used to control and treat COVID-19 infection (Balmeh et al., 2020).

The studies in which bioactive components were examined for both the treatment and supportive treatment of the new COVID-19 viral infection reported that plants such as G. glabra, Allium sativum, Althea officinalis, and Panax ginseng can support the immune system together with thyme (Jalali et al., 2021). It has been revealed that Thymus *Vulgaris*, due to its antioxidant properties, has an immunosuppressive effect in respiratory diseases, so can reduce the symptoms of coronavirus disease (Sardari et al., 2021), and Salvia officinalis can also be used for a strong immune system for COVID-19 (Reddy et al., 2021).

#### 2. CULTIVATION OF SAGE **AND** THYME IN **ARID CONDITIONS**

The main factor leading to starvation in barren or semi-barren places is the dryness or reduction of soil and groundwater and the resulting famine. Barrenness occurs due to a lack of precipitation and moisture in the region, as well as due to potential evaporation and evapotranspiration. It is inevitable that agricultural and livestock products will be adversely affected in unproductive soils due to the emergence of salinization in agricultural soils in arid regions caused by climate change. Therefore, drought-resistant plants should be cultivated in arid regions where precipitation is low and the soil loses moisture due to high temperatures.

S. officinalis grows well in dry meadows and steppes at an altitude of up to 750 m and in hilly, clay soil (Sharma et al., 2019). In sandy soils, plant nutrients and water holding capacity are low. However, sage and

thyme are resistant to dry conditions, so they also grow well on sandy soils. However, sage and thyme are perennial woody plants grown in mountainous and sloping areas because they can prevent erosion thanks to root systems that can adapt to various environmental conditions in well-drained soil, which are abundantly sunny, tolerant of drought and high temperatures (Alatzidis, 2019). Mehalaine & Chenchouni (2020) stated that the production of essential oils obtained from *Thymus vulgaris* grown in sandy soil is excessive, but less in clay soil, and moderate in calcareous soil. In sandy soil, the development of *Salvia officinalis* treated with worm humus applications was greater compared to those treated with compost and controls (without any additives) (El-Haddad et al., 2020).

Medical and aromatic plants belonging to the family of *Lamiaceae* activate their defense systems by increasing the metabolic mechanisms of phenolics, flavonoids, terpenoids, stress hormones, etc. to adapt to stress. A study examining the volatile yield of *Thymus carmanicus*, native to the semi-arid regions of Iran, under the water regimes of 20-50% and 80%, reported that the plant must be watered in a 20% water regime for maximum essential oil yield and that the main component of this species is carvacrol (Bahreininejad et al., 2014). In a study examining the effects of drought stress on *S. sinaloensis*, plants were grown in control conditions without semi-irrigation (moderate drought) or irrigation. As a result, it has been reported that carotenoid content is not affected by water regimens, the percentage of phenols and flavonoids increases in plants under both stress conditions, and *S.* 

sinaloensis can be preferred as a drought-resistant plant with high secondary metabolites by saving irrigation water (Caser et al., 2018). Bistgani et al., (2019) studied the physiological properties and antioxidant activities of *Thymus vulgaris* and *Thymus daenensis* plants in irrigation containing 0, 30, 60, and 90 mM NaCl. In the study, plant dry matter production at a dose of 90 mM NaCl was about 40% in T. vulgaris and 39% in T. daenensis, which were decreased significantly compared to the control, but an increase in leaf flavonoid content of 38.6% and 36.6% were observed after 60 and 90 mM NaCl administration, respectively. Salinity stress significantly reduced the yield of both species, the phenolic content and antioxidant capacity of thyme plants improved. It was found that *T. daenensis* was similar to *T.* vulgaris in terms of tolerance to high levels of salinity stress, and both species were moderately tolerant to severe salt stress. Bistgani et al., (2019) stated that *Thymus vulgaris* L. underwent significant reductions in the shoot, root, wet and dry weight under water stress, while there was a significant increase in shoot/root dry matter ratio, and this thyme species developed better in water scarcity. When the growth and antioxidant activities of 'Isfahan', 'Violet Queen' and 'Rose Queen' sage cultivars under water stress were investigated, the development of leaves, roots, and shoots in the 'Isfahan' variety was found to be good, and the content of proline, total phenol, and flavonoids of all varieties increased in drought conditions (Bayat and Moghadam 2019). The essential oil content of Thyme (Thymus spp.) is affected by different environmental factors including drought. In a study investigating soil moisture, water potential, shoot dry weight, photosynthetic ratio,

essential oil composition, etc. in water scarcity, plants were given water at intervals of 4 days until they were withering. The study reported that drought-tolerant plants exhibited similar photosynthesis rates and growth compared to control plants (Mahdavi et al., 2020).

Coating seeds with biostimulants are among the promising approaches to increase crop tolerance to drought stress in crop production. In one study, germination and seedling growth and water and nutrient status of plants were improved in wheat seeds coated with thyme oil grown in well-irrigated conditions. Coating seeds with thyme oil in stress conditions has optimized water intake through deep rooting in some varieties, had a positive effect on water stress resistance by maintaining better carbon assimilation and nitrogen intake. Overall, it has been stated that applying thyme oil to seeds could be an alternative approach to enhance drought resistance (Ben-Jabeur et al., 2019). It is obvious that current research results can be assessed in organic grain farming. Photosynthetic daily light integral and temperature environmental factors that profoundly influence plant growth and development. (Moccaldi ve Runkle 2007). Applying different sources of potassium to Salvia farinacea leaves grown in partial shade (50%) increased plant size and leaf area, while full solar conditions increased the number of stems, root, leaves, branches, and blooms, fresh and dry weight of leaves, as well as chlorophyll, carbohydrate, and mineral contents (Heikal, 2017). Rezai et al., (2018) studied to find the optimum light intensity required for sage development in the semi-arid areas and determined plant morphology and photosynthetic capacity at full light

(0% shadow), 30%, 50%, and 70% shadow levels. In the study, they reported reduced essential oil content and plant growth in the shade over 30%, maximum leaf area in the shade of 70% but increased chlorophyll and carotenoid content in line with decreased light intensity, and the optimal light request was 30% shade.

#### 3. THE USE OF SAGE AND THYME IN SOIL REMEDIATION

Excessive irrigation, widespread use of chemical fertilizers, and global warming result in soil losses, accumulation of nitrate in crops, growth and productivity problems in plants, as well as poor quality plant products that negatively affect human health. In organic agriculture, practices related to soil management, relationships between soil, plant, and soil organisms are examined, and soil remediation is focused on. Therefore, the usefulness and intake of plant nutrients, as well as ecosystem-friendly solutions are found. In recent years, research has been conducted on the use of crop waste, farm fertilizer, green fertilizer, and plant-beneficial microorganisms as alternatives to chemical use in organic farming.

Some aromatic plant species can tolerate adverse environmental conditions and have been recommended to be grown in contaminated, eroded, and moisture deficient soils. Aromatic plants are grown as soil cover in fruit gardens. Cover crops are used to control biodiversity and habitat quality, pest and disease control, land losses. In a study where thyme was planted between vineyard rows for weed and erosion control in a vineyard cultivated on steep and rocky soils according to organic

principles, the effects of growing aromatic plants on soil properties were investigated, and thus soil nitrate and soil ammonium (NH<sub>4</sub>-N) were found to decrease in the upper soil layer due to thyme cultivation (Dittrich et al., 2021).

The presence of high concentrations of trace elements in soils can negatively impact not only microbial diversity and flora composition, but also plant formation and growth. The use of mineral, organic or microbial exogenous amendments can support plant growth and health. Therefore, microbial inoculants such as arbuscular mycorrhizal fungi (AMF) or ectomycorrhizal fungi are becoming important. The planting of sage (*Salvia sclarea L.*) and mycorrhizal vaccination in trace element-polluted soil has been stated to significantly enhance total fungal flora composition (Raveau et al., 2021). The outcome of this research could be a guide in improving inefficient soils in organic farming.

Fast-growing, deep-rooted, and high-biomass metal stabilizing plants that can produce high-value economic products have huge potential. The use of medicinal and aromatic plants and their associated microorganisms is beneficial in organic farming for reestablishing degraded areas, and an increase in soil organic matter content is ensured due to the decomposition of plant residues, thus improving the physical and chemical properties of the soil, resulting in increased plant growth. Medical and aromatic plants that grow in soils contaminated with heavy metals become safe-to-use products because they are not significantly affected by heavy metals. Again, these plants have a beneficial effect

on soil quality by controlling erosion and reducing surface runoff when grown as vegetation cover (Ait Elallem et al., 2021).

# 4. THE USE OF SAGE AND THYME AS AN ANTIMICROBIAL **AGENT**

Plant-derived substances have an important place in organic farming in terms of their positive impact on human and environmental health. In particular, medical and aromatic plant extracts without toxic effects serve as antimicrobial and pesticides in promoting plant defense against plant disease and pests. Therefore, considering medicinal and aromatic plants, special emphasis is given to studies on their usability in achieving efficient and quality products.

Various pathogens, including bacteria, fungi, and yeasts, are commonly found in composts made up of green waste, fertilizer, sewage sludge, and solid wastes. Medical and aromatic plants are useful in preventing the proliferation of pathogens in composts. Essential oil and active ingredients affect the microbial flora in composts. Sage oil is known to have antifungal activities against both yeast and fungi types due to the fact that it contains thujone, 1,8-cineole, camphor, borneol, and limonene. Thyme oil consists of terpenes, terpene alcohols, aldehydes, phenolic derivatives, ketones, esters, and ethers. Besides this, the main ingredients are carvacrol and thymol. Thyme essential oil, carvacrol, thymol, and p-cymene also have remarkable antifungal activity against various types of mould and yeast (Greff et al., 2021).

Grains contain numerous microorganisms that impair their nutritional value and are dangerous to human and animal health. Synthetic pesticides have an important role in preserving the crop. But widespread use of pesticides has led to the development of pest resistance, new pest outbreaks, and harmful effects on the environment. There is therefore a need to develop new fungicides/preservatives whose performance is improved and also environmentally friendly in nature. Low doses of essential oils, such as thyme oil, have an antifungal effect. This can be part of organic production and extend shelf life (Střelková et al., 2021). Again, the essential oil of sage crops can be used as an alternative to synthetic pesticides to control post-harvest diseases and extend the shelf life of fruit crops (Samara et al., 2021), which is an important approach for organic farming.

Synthetic fungicides used to counter the reductions in yields and quality caused by fungi and disease in legumes that are important in human and animal nutrition causes environmental and health concerns, besides pathogens resistant to fungicides. Studies investigating organic herbal solutions to fungi have revealed that thyme essential oil inhibits all pathogenic fungal development (Parikh et al., 2021), again controls bacterial wilt of potatoes (Sallam et al., 2021), and has the potential to be used as antibiotics (Oluoch et al., 2021). Moreover, it has been determined that the disease severity of rust disease is reduced by spraying and applying thyme plant extract to increase the resistance of pods grown in greenhouse and field conditions. (El- Fawy et al., 2021).

Plant-based natural herbicides are used in organic farming as an alternative to synthetic herbicides for weed control. In this context, allelopathic plant extracts that can produce second metabolites can be harnessed. Sage and thyme essential oils from Lamiaceae family species have the potential as bioherbicide (De Mastro et al., 2021).

#### **CONCLUSION**

Sage and thyme have been consumed as spices, teas, and oils throughout history, taking advantage of their antifungal, antiviral, and antibacterial effects to protect and treat many diseases as medicinal plants. Today, antiviral plants such as sage and thyme, which contain antioxidants, should be used to combat the corona virus pandemic caused by the COVID-19 virus. Antiviral plants not only fight viral infections, boost the immune system and work as a natural medicine against flu, but also have significant benefits against the diseases of the heart and digestive system, as well as, chronic diseases.

Climate changes, such as diversification of climates, sudden warming, and cooling of the atmosphere, declining natural resources, are major issues of concern to the world in terms of negative impacts and are expected to continue to accelerate in the near future. The impact of climate change on agriculture, which is sustained on a natural basis, is excessive. A number of measures must be taken in the organic farming system against global climate changes, the agricultural environment, drought, desertification.

Sage and thyme, which can grow on their own thanks to their natural properties and adaptation areas, have important features. These plants can adapt to all kinds of climatic conditions and can be grown naturally in almost all of the soil and all of the season. Sage and thyme, which thrive perennially in cold climate regions, can survive until spring.

Sage and thyme develop well in well-drained soils, sunny conditions, hot and dry climates. Sandy soils have low water holding capacity and low moisture-holding properties. So nutrients in soil move away from the soil easily and quickly because of drainage, therefore sage and thyme that don't need a lot of plant nutrient elements for growth and development should be grown in such conditions. Thus, sage and thyme farming, which adapts to dry conditions, becomes important. Both plants can be grown on inefficient soils to prevent water scarcity and erosion caused by climate change. Because they have a dense root system, soil erosion can be prevented by growing in prone areas and mountainous areas. Thus, possible alternatives and opportunities for organic farming can be created.

Selecting suitable areas on the world where strategically important medical and aromatic plants can be grown in today's climate conditions could be effective in preventing potential adverse effects to soil and water resources in the future. In the organic farming model, important medical and aromatic plants such as sage and thyme should be heavily given importance to mitigate adverse

effects resulting from over-used chemicals such as fertilizers and pharmaceuticals in traditional agriculture.

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#### **CHAPTER 11**

# INSECTICIDAL ACTIVITIES OF DIFFERENT ESSENTIAL OILS ON RHYZOPERTHA DOMINICA (F.) (COLEOPTERA: BOSTRICHIDAE) ADULTS

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#### **INTRODUCTION**

The world is facing a growing population and its increasing food requirements in todays. Sustainable agricultural production, increased productivity from the unit-level and the minimization of crop losses from agricultural production are required to off-set this population groving. Sustainability is achieved through cultural practices such as selection of varieties, irrigation and fertilization, and protection from harmful plant diseases and pests (Kotan et al., 2008; Toprak et al., 2020). Therefore, organic farming comes into fore. Organic agriculture; is a holistic production management system which promotes and enhances agro-ecosystem health, including biodiversity, biological cycles, and soil biological activity (https://www.fao.org/organicag/oafaq/oa-faq1/en/). For this reason, organic agriculture is of great importance for life, as it protects human and environmental health. In addition, healthy and safe storage of organic products is of particular importance for human and environmental health. Cereals have an important role for feeding to this growing population and animals in not only Turkey but also the world (Togay et al., 2008). They are the raw material of daily feeding in human and animal nutrition in the world, which is constantly changing and developing. Due to their prosperous source of nutritious elements, the cereals are the most consumed food worldwide as fresh and dried. In addition, they are widely used in different industrial productions such as malt, pasta, flour, etc.. Therefore, their productions are among the most important agricultural activities practiced in worldwide (Toprak et al., 2020). However, there are many insect pests causing to decrease the quality and edible quality of the cereals especially during storage.

Among them, the lesser grain borer (LGB), Rhyzopertha dominica (Fabricus, 1792) (Coleoptera: Bostrichidae), is a notable coleopteran pest of various stored cereal grains that are the basic sources of energy and food for human and animals in most countries of the world. It is believed that the origin of the LGB is India on dead wood. It had been known as "wood bug" until 1911 (Chittenden, 1911), but this species has become a potential problem in many different regions of the world, where grain is produced and stored because of its adaptability to feed on cereal grains as well as other substrates containing starch (Jia et al., 2008; Mahroof et al., 2010). Adults are very strong flier and can easily pass from one storage to another (Hagstrum, 2001). The lifecycle consists of four or more months including; egg, larva, pupa and adult stages. A female may lay more than 500 eggs in clusters or solely on the inner surfaces of grain kernels (Kucerová and Stejskal, 2008; Ozkaya et al., 2009). The LGB's larvae and adults feed on entirety, well and solid grains. They can lead to seriously damages to the wheat grains they feed on. They cause damages by feeding in both the germ and endosperm, and can also reduce grain quality by leaving pheromone odor, and production of frass (Feroni et al., 2004). The LGB larvae develop inside the grain. Therefore, control of this pest in stored grain by using the insecticides is quite difficult. Furthermore, randomly use of syntetic chemical products to prevent losses in stored products can be toxic to other organisms and result in the evolution of insecticide

resistant individuals. Also, these syntetic chemicals can lead to toxic residues and environmental pollution (Cox, 2004; Koul et al., 2008). Thus, the researchers have focused on the obtaining of alternative substances having insecticidal effects that are relatively less harmful to non-target organisms and the environment (Isman, 2006; Sahaf & Moharramipour, 2008). It was reported that the essential oils (EOs) obtained from different plant species could be used as attractive, repellent, fumigants or contact effective insecticides against the LGB (Shaaya et al., 1991; Wu et al., 2009; Hosseini et al., 2013; Dhen et al., 2014; Ustuner et al., 2018; Gokturk et al., 2020, 2021) and other stored product insects (Papachristos & Stamopoulos, 2002; Yildirim et al., 2005; Semiz et al., 2006; Farhana et al., 2006; Bittner et al., 2008; Ayvaz et al., 2010; Pathipati, 2012; Selimoglu et al., 2015; Usanmaz Bozhüyük et al., 2016; Rquez et al., 2019). For the mentioned reasons, many researhers focus on PEOs for their broad spectrum pest control ingredients nowadays.

The main objective of the present study was to detect the toxicity of the EOs extracted from 11 different plant species, *S. cilicica, S. cuneifolia, S. hortensis, S. spicigera, S. thymbra, S. montana, O. vulgare, O. onites, O. syriacum, Th. spicata* and *Th. Sipyleus*, against the LGB (*R. dominica*) adults, an important stored-product pest, under in vitro conditions.

#### 1. MATERIAL AND METHODS

#### 1.1. Plant Materials and Obtaining Essential Oils

The tested plant species (Satureja cilicica L., S. cuneifolia Ten., S. hortensis L., S. spicigera (C. Koch) Boiss., S. thymbra L., S. montana L., Origanum vulgare L., O. onites L., O. syriacum L., Thymbra spicata L., and Thymus sipyleus Boiss.) (Lamiaceae) were gathered from the Northeast Anatolian area of Turkey at the flowering periods between June and August in 2018-2019, in the study. The plant materials were dried in shade. After that, the erial parts of these dried materials were powdered and sifted out with a sieve prior to extraction. The sifted out materials (500g) were processed to hydrodistillation (plant materials in welding water) using a Neo-Clevengerequipment for 3-4 hrs. The oil yields of S. cilicica, S. cuneifolia, S. hortensis, S. spicigera, S. thymbra, S. montana, O. hirtum, O. onites, O. syriacum, T. spicata and T. sipyleus were 1.20%, 1.5%, 2.3%, 1.56%, 1.17%, 1.28%, 2.2%, 0.63%, 4.0%, 2.4% and 0.98% (w/w, dry weight basis), respectively. The EOs were seared over anhydrous Na<sub>2</sub>SO<sub>4</sub> and conserved under N<sub>2</sub> in a small imperforatedbottle at 4 °C until used for toxicity bioassays.

# 1.2. Main Chemical Compositions of The Tested Essential Oils

The main chemical compositions of the EOs obtained from aerial parts of the test plant species (S. cilicica, S. cuneifolia, S. hortensis, S. spicigera, S. thymbra, S. montana, O. vulgare, O. onites, O. syriacum, T. spicata and T. sipyleus) were given in Table 1 with the literature.

Thymol, Carvacrol, Geranial and  $\gamma$  –Amorphene were recorded to be the main components of these PEOs Table 1.

### 1.3. Biological Material

The LGB adults used in the study were provided from the storage houses and flour mills in Mugla province (Fethiye). The insect adults were reprocuded at Mugla Sitki Kocman University, F.A.S.M.K. Vocational School, Department of Environmental Protection Technologies, Fethiye, Mugla, Turkey and fed on the mixed of the cracked grains, bran, flour and crubs at 25±1°C, 65±5% RH, 16 h:8 h (L:D) without subjection to any pesticide up to test times. The insect adults were observed a weekly. For the toxicity testing of the EOs against to the 5-6 days old adult insects, 20 insect adults and enough the mixed of the cracked grains, bran, flour and crubs were placed in Petri dishes (9 x 1.5 cm). All test methods were achieved under the same conditions as expicated above.

# 1.4. Bioassays

In order to determine the fumigant toxicities at 5, 10 and 20 μL petri<sup>-1</sup> of 11 different PEOs (*S. cilicica*, *S. cuneifolia*, *S. hortensis*, *S. spicigera*, *S. thymbra*, *S. montana*, *O. vulgare*, *O. onites*, *O. syriacum*, *T. spicata* and *T. sipyleus*), A No:1 Whatman filter paper was fixated onto the inner top of the Petri dishes. After, 5-6 day-old 20 adults of the LGB were put in each Petri dish. The enough mixed of wheat grains, breaked grains, pure flour were added for feeding them. The PEOs were maked suck into the paper the inner top of the Petri dishes. This prevented

directly contacting between the PEOs and the tested insects. Each petri dish was closed with a lid separately and carried to an incubator. Then, they were upholded under the test conditions (at 25±1°C, 65±5% RH, and in 16 h:8 h (L:D)) for 96 h. The insect mortalities in each Petri dishes were regarded at the 24<sup>th</sup>, 48<sup>th</sup>, 72<sup>nd</sup> and 96<sup>th</sup> hrs. For the insects tested, the other Petri dish treated with sterile water solely was employed as the control. Each treatment was repeated with three replications (also, three sub-replications for each concentration and exposure time combination). The toxicities of the PEOs were indicated as percentage mean mortalities of the insects.

## 1.5. Statistical Analyses

Mortality counts were predicated to oneway variance analyse (ANOVA), using SPSS 17.0 software package. Mortalities were established as mean (%)  $\pm$  SE. Differences between means were tested through Duncan test and values with P< 0.05 were taken into consideration substantially different. The LC (LC<sub>50</sub> and LC<sub>90</sub>) values were estimated according to (Finney, 1971). Probit analysis of dosemortality data was coordinated with calculate the LC (LC<sub>50</sub> and LC<sub>90</sub>) values of related 95% assurance limits for each exposure using the Probit Analysis Program (EPA).

#### 2. RESULTS AND DISCUSSION

#### 2.1. Main Chemical Compositions of The PEOs

The main chemical components of the tested EOs from Satureja cilicica L., S. cuneifolia Ten., S. hortensis L., S. spicigera (C. Koch) Boiss., S. thymbra, S. montana, Origanum vulgare, O. onites, O. syriacum, Thymbra spicata L., and Thymus sipyleus Boiss. (Lamiaceae) with literature were given in Table 1, separately. Thymol, Carvacrol, Geranial and y–Amorphene were recorded to be the main components of these PEOs (Bayan et al., 2017; Usanmaz Bozhüyük et al., 2018; Duran & Kaya, 2018; Tasdemir et al., 2019; Bozkurt et al., 2020; Gonçalves et al., 2021). Thymol (68.9%), p-Cymene (7.79%) and Borneol (2.95%) were determined as the main components for S. *cilicica*. Similarly, y – Amorphene (35.47%), Germacrene-D (17.63%) and 6,9-Guaiadiene (11.67%) for S. cuneifolia; Thymol (72.18%), p-Cymene (9.74%) and γ –Terpinene (7.61%) for S. hortensis; Carvacrol (90.25%; 71.31%), p-Cymene (4.12%; 6.06%) and  $\gamma$  -Terpinene ( 2.58%; 11.87%) for S. spicigera and S. montana, respectively; Carvacrol (57.13%), p-Cymene (21.95%) and Thymol (7.98%) for S. thymbra were found as main compositions (Usanmaz Bozhüyük et al., 2018).

**Table 1.** Main components of the essential oils of test plants

Essential oils	Main components (%)
	Thymol (68.9%)
Satureja cilicica	p-Cymene (7.79%)
	Borneol (2.95%)
	20111601 (21,50,70)
	$\gamma$ –Amorphene (35.47%)
Satureja cuneifolia	Germacrene-D (17.63%)
	6,9-Guaiadiene (11.67%)
	Th1 (72.199/)
	Thymol (72.18%)
Satureja hortensis	p-Cymene (9.74%)
	γ –Terpinene (7.61%)
	Carvacrol (90.25%)
Satureja spicigera	p-Cymene (4.12%)
J	γ-Terpinene (2.58%)
	Compagned (57, 120/)
C -4	Carvacrol (57.13%)
Satureja thymbra	p-Cymene (21.95%)
	Thymol (7.98%)
	Carvacrol (71.31%)
Satureja montana	γ –Terpinene (11.87%)
	p-Cymene (6.06%)
	Carvacrol (67.67%)
Origanum vulgare	o-cymene (11.60%)
	$\gamma$ -Terpinene (7.45%)
	Terpinene (7.1570)
	Carvacrol (70.6%)
Origanum onites	Linalool (9.7%)
	p-Cymene (7.0%)
	Thymol (42.18%)
Origanum syriacum	Carvacrol (33.95%)
	Cymene (8.87%)
	- , ( ,
	Carvacrol (78.53%)
Thymbra spicata	γ –Terpinene (10. 42%)
	o-Cymene (5.5%)
	Geranial (13.72%)
Thymus sipyleus	Carvacrol (11.06%)
Inymus sipyteus	Thymol (10.17%)
	111y11101 (10.17/0)

In addition, Carvacrol (67.67%), o-cymene (11.60%) and  $\gamma$  –Terpinene (7.45%) for *O. vulgare* (Gonçalves et al., 2021); Carvacrol (70.6%), Linalool (9.7%) and p-Cymene (7.0%) for *O. onites* (Tasdemir et al., 2019); Thymol (42.18%), Carvacrol (33.95%) and Cymene (8.87%) for *O. syriacum* (Duran & Kaya, 2018); Carvacrol (78.53%),  $\gamma$  –Terpinene (10. 42%) and o-cymene (5.5%) for *T. spicata* (Bayan et al., 2017); and Geranial (13.72%), Carvacrol (11.06%) and Thymol (10.17%) for *T. sipyleus* (Bozkurt et al., 2020) were recorded to be main components.

#### 2.2. Insecticidal Activity

In this study, the insecticidal activities of 11 different PEOs were evaluated on the adults of the LGB adults and the results are summarized in Table 2. The results indicated that the tested oils had toxicities on the LGB adults in comparison with controls. The analysis of variance proves that the affects on the toxicity rate of the LGB adults are highly substantial on the main of doses and exposure time (Table 2). The mortalities (%) of the LGB are shown in Table 2 at the used doses (5, 10 and 20 µLpetri<sup>-1</sup>). The maximum toxicity was established at an exposure time of 96 h, while the minimum toxicity recorded at an exposure time of 24 h (Table 2). Higher doses and longer exposure times resulted in the most mortality on the LGB adults. Analysis of varience showed that the efficacies of the 11 PEOs, doses, exposure

times and their interactions on the mortalities of the adult insects were statistically very meaningful (Tables 2 and 3) (P< 0.05).

The treatment of S. cilicica, S. hortensis, S. montana, S. spicigera, S. thymbra and Th. sipyleus EOs led to higher mortalities against the LGB adults, but that of O. vulgare, O. onites, O. syriacum, S. cuneifolia and Th. spicata EOs caused more less mortalities. As it can be seen from the Table 2, all the EOs were toxic against the LGB adults, but toxicities of the EOs were different from plant to plant. S. cilicica and S. hortensis EOs were more toxic against the LGB adults in comparison to other EOs (Table 2). All the doses of *S. cilicica* and *S. hortensis* EOs showed 100% mortality on the LGB adults after 96 h of exposure. The emergence of early fumigant effects of the EOs were very important. Most EOs caused between 45 and 96.6% mortality on the LGB adults, at 20 µLpetri<sup>-1</sup> after 24 h exposure. S. montana EO attained 100 % of mortality at 20 µLpetri-1 after 48 h, incontrast with S. thymbra which after 72 h of treatments, at the same dose (at 20 µLPetri<sup>-1</sup>) caused 100 % mortality. The positive correlations were observed between the treatment dose and insecticidal effect; passed time and toxicity (Table 2). The highest mortality rate (100%) were obtained after 72 and 96 hrs. After 72 h, 100% mortality in the PEOs of S. cilicica, S. hortensis and S. montana at the doses of 10 and 20 µLpetri<sup>-1</sup>, and in the same percent of mortality (100%) in S. thymbra but only at 20 µLpetri<sup>-1</sup> were observed, whereas after 96 h of the treatments, 100% mortalities of the insect were recorded and it was like this; 100% mortality caused by the EOs of S. montana, S. spicigera, S. thymbra and Th. sipyleusat 10 and

20  $\mu$ Lpetri<sup>-1</sup> and 100% at 20  $\mu$ Lpetri<sup>-1</sup> doses of *O. vulgare, O. onites, O. syriacum, S. cuneifolia* and *Th. spicata* EOs. These results show that the 11 PEOs had varying degrees of fumigant effects on the LGB adults as the toxicity increased with increasing dose and time. Besides, *S. cilicica, S. hortensis* and *S. spicigera* EOs were found to be the most effective mortality with rate of 100%, at 5, 10 and 20  $\mu$ Lpetri<sup>-1</sup> doses at the 96 h of the exposure (Table 2).

**Table 2.** Mortality rates after 24, 48, 72 and 96 hrs against *R. dominica* adults at doses of 5, 10 and 20 uL petri<sup>-1</sup> of eleven plant essential oils

Rhyzopertha dominica								
	Exposure time (h) and Mortality (%) <sup>a</sup>							
Essential oils	<b>Dose</b>	<u>24<sup>b</sup></u>	<u>48<sup>b</sup></u>	<u>72<sup>b</sup></u>	<u>96<sup>b</sup></u>			
	5	$41.1 \pm 1.66$	$81.6 \pm 1.66$	$96.6 \pm 1.66$	$100 \pm 0.0$			
Satureja	3	$\begin{array}{c} 41.1 \pm 1.00 \\ \text{efg} \end{array}$	81.0 ± 1.00 1jklm	90.0 ± 1.00 ghi	$100 \pm 0.0$			
cilicica	10	$41.6 \pm 4.40$	$83.3 \pm 1.66$	$100 \pm 0.0$ 1	$100 \pm 0.0$			
cincica	10	efg	jklm	100 ± 0.0 1	c c			
	20	$65.0 \pm 2.88$	$86.6 \pm 1.66$	$100 \pm 0.0$ 1	$100 \pm 0.0$			
		kl	lmno		c			
	5	$25.0 \pm 2.88$	$43.3 \pm 4.40$	$83.3 \pm 4.40$	$95.5 \pm$			
Satureja		b	b	bc	2.88 b			
cuneifolia	10	$38.3 \pm 1.66$	$55.5 \pm 2.88 \text{ c}$	$88.3 \pm 4.40$	98.3 ±			
	•	def	666.440	cde	1.66 bc			
	20	$45.0 \pm 2.88$	$66.6 \pm 4.40$	$95.0 \pm 2.88$	$100 \pm 0.0$			
		fgh	def	fghı	С			
	5	$91.1 \pm 1.66$	$96.6 \pm 1.66$	$98.3 \pm 1.66$	$100 \pm 0.0$			
Satureja	C	0	pr	hı	c c			
hortensis	10	$93.3 \pm 1.66$	$98.3 \pm 1.66$	$100 \pm 0.0$ 1	$100 \pm 0.0$			
		О	pr		c			
	20	$96.6 \pm 3.33$	$98.3 \pm 1.66$	$100 \pm 0.0~\text{1}$	$100 \pm 0.0c$			
		О	pr					
	5	$91.1 \pm 1.66$	$96.6 \pm 1.66$	$98.3 \pm 1.66$	$100 \pm 0.0$			
g .	10	0	pr	h1	C			
Satureja	10	$93.3 \pm 1.66$	$98.3 \pm 1.66$	$100 \pm 0.0$ 1	$100 \pm 0.0$			
spicigera	20	0 $96.6 \pm 3.33$	pr 98.3 ± 1.66	$100 \pm 0.0_{\ 1}$	$\begin{matrix} c \\ 100 \pm 0.0c \end{matrix}$			
	20	$90.0 \pm 3.33$	$98.3 \pm 1.00$ pr	100 ± 0.0 1	100 ± 0.00			
		U	Pι					

	5	$63.3 \pm 1.66$	$83.3 \pm 1.66$	$93.3 \pm 4.40$	$96.6 \pm$
Satureja		kl	jklm	efgh	3.33 bc
thymbra	10	$80.0 \pm 2.88$	$90.0 \pm 2.88$	$96.6 \pm 1.66$	$100\pm0.0$
inymora	10	n	noöp	ghı	c
	20			$100 \pm 0.0$ 1	
	20	$95.0 \pm 5.0$	$98.3 \pm 1.66$	$100 \pm 0.01$	$100\pm0.0$
		О	pr		c
	5	$66.6 \pm 6.01$	$88.3 \pm 4.40$	$98.3 \pm 1.66$	$98.8 \pm$
Satureja			mnoö	hı	1.66 bc
montana	10	$90.0 \pm 0.0$	$95.0 \pm 0.0$	$100 \pm 0.0$ 1	$100 \pm 0.0$
		0	öpr		c
	20	$93.3 \pm 4.40$	$100 \pm 0.0 \text{ r}$	$100 \pm 0.0$ 1	$100 \pm 0.0$
		0	100 = 0.01	100 = 0.01	c
		O			C
	5	25.0 + 5.0	62 2 + 4 40	950 + 200	05.0
0 :	3	$35.0 \pm 5.0$	$63.3 \pm 4.40$	$85.0 \pm 2.88$	95.0 ±
Origanum	4.0	cde	d	bcd	2.88 b
vulgare	10	$41.1 \pm 1.66$	$71.6 \pm 1.66$	$90.0 \pm 2.88$	98.3 ±
		efg	efgh	def	1.66 bc
	20	$53.3 \pm 1.66$	$83.3 \pm 1.66$	$95.0 \pm 0.0$	$100 \pm 0.0$
		1j	jklm	fghı	c
	5	$36.6 \pm 3.33$	$73.3 \pm 1.66$	$90.0 \pm 5.0$	$96.6 \pm$
Origanum		cdef	fghı	def	1.66 bc
onites	10	$41.1 \pm 1.66$	$76.6 \pm 1.66$	$88.3 \pm 1.66$	$98.3 \pm$
0711105		efg	hıjk	cde	1.66 bc
	20	$51.6 \pm 1.66$	$83.3 \pm 3.33$	$95.0 \pm 0.0$	$100 \pm 0.0$
	20	h1j	jklm	fgh1	c c
		1111	JKIIII	igiii	C
	5	$30.0 \pm 5.0$	$65.0 \pm 2.88$	016 + 166	96.6 ±
0 :	3			$81.6 \pm 1.66$	
Origanum		bc	de	b	1.66 bc
syriacum	10	$40.0\pm5.77$	$80.0\pm2.88$	$95.0 \pm 2.88$	$98.3 \pm$
		efg	ıjklm	fghı	1.66 bc
	20	$58.3 \pm 1.66$	$86.6 \pm 1.66$	$98.3 \pm 1.66$	$100 \pm 0.0$
		jk	lmno	hı	c
	5	$36.6 \pm 1.66$	$66.6 \pm 1.66$	$85.0 \pm 2.88$	$96.6 \pm$
Thymbra		cdef	def	bcd	1.66 bc
spicata	10	$40.0 \pm 2.88$	$75.0 \pm 2.88$	$93.3 \pm 1.66$	98.3 ±
spicara	10	efg	ghij	efgh	1.66 bc
	20	$55.0 \pm 2.88$	$85.0 \pm 5.0$	$96.6 \pm 1.66$	$1.00 \text{ bc}$ $100 \pm 0.0$
	20				
		1J	klm	ghı	С
	-	21 6 + 2 22	60.2 + 4.40	00.2 + 4.40	00.2
TI.	5	$31.6 \pm 3.33$	$68.3 \pm 4.40$	$88.3 \pm 4.40$	98.3 ±
Thymus		bcd	def	cde	1.66 bc
sipyleus	10	$41.6 \pm 3.33$	$78.3 \pm 1.66$	$95.0 \pm 0.0$	$100\pm0.0$
		efg	hıjkl	fghı	c

	20	48.3 ± 1.66 gh1	80.0 ± 10.0 1jklm	98.3 ± 1.66 hı	100 ± 0.0 c
Control (non-treated)	20	$0.0 \pm 0.0 \; a$	$0.0 \pm 0.0 \; a$	$0.0\pm0.0\;a$	$0.0 \pm 0.0$ a

 $<sup>{}^{</sup>a}Mean \pm SE$  of three replicates, each set-up with 20 adults

When LC<sub>50</sub> and LC<sub>90</sub> values after 96 h treatment of 11 PEOs were compared for their effects on the LGB adults, the most toxic EOs based on LC<sub>50</sub> and LC<sub>90</sub> values were determined for *O. onites*, *O. syriacum* and *Th. spicata* (0.435 and 2.500 μLinsect<sup>-1</sup>) PEOs, respectively. However, the lowest toxic EOs after 96 h, based on LC<sub>50</sub> and LC<sub>90</sub> values, were with *S. spicigera* and *S. thymbra* PEOs (1.910 and 3.755 μLinsect<sup>-1</sup>) with the LGB adults, respectively (Table 3). In addition, after 96 h, while the LC<sub>50</sub> value of *S. cilicia*, *S. hortensis*, *S. montana* and *Th. sipylus* PEOs was recorded as 1.387 μLinsect<sup>-1</sup>, the LC<sub>90</sub> value was determined as 3.013 μLinsect<sup>-1</sup> for the same PEOs. However, LC<sub>50</sub> and LC<sub>90</sub> values, after 96 h, of *S. cuneifolia* and *O. vulgare* EOs were observed as 0.861 and 3.468 μLinsect<sup>-1</sup>, respectively (Table 3). All of the PEOs gave meaningful mortalities. However, there was no mortality in the controls of each treatment (except, 96 h, 3.33%) (Tables 2 and 3).

<sup>&</sup>lt;sup>b</sup>Exposure time (h)

Values followed by different letters in the same column differ significantly at \*P< 0.05

Table 3.	$LC_{50}$	and	$LC_{90}$	toxicity	values	of	eleven	plant	essential	oils	against	R.
dominica	adults	3										

Essential oils	<u>LC50</u> <sup>a</sup>	<u>LC90</u> <u>b</u>	$(\lambda^2)^c$	$\underline{Slope \pm S.E.^d}$
Satureja cilicica	1.387	3.013	2.020	$3.804 \pm 5.366$
Satureja cuneifolia	0.861	3.468	5.031	$2.118 \pm 0.138$
Satureja hortensis	1.387	3.013	2.020	$3.804 \pm 5.366$
Satureja spicigera	1.910	3.755	1.066	$4.364 \pm 4.523$
Satureja thymbra	1.910	3.755	4.108	$4.364 \pm 4.523$
Satureja montana	1.387	3.013	2.020	$3.804 \pm 5.366$
Origanum vulgare	0.861	3.468	5.031	$2.118\pm1.278$
Origanum onites	0.435	2.500	4.425	$1.687 \pm 1.271$
Origanum syriacum	0.435	2.500	4.425	$1.687 \pm 1.271$

<sup>&</sup>lt;sup>a</sup> The lethal concentration causing 50% mortality after 96 h

In the previous studies, some PEOs led to important toxicity on the stored-product pests, such as the LGB, and it was also stated that the toxicities of each PEO varied importantly among the insect species (Wu et al., 2009). In their study, Lee et al. (2004) reported that six PEOs belonging to the family Myrtaceae had potential fumigant toxicities against a major stored-grain insect, the LGB adults. Hosseini et al. (2013) determine that Salvia leriifolia (Bent) (Lamiaceae) EO had a

<sup>&</sup>lt;sup>b</sup> The lethal concentration causing 90% mortality after 96 h

<sup>&</sup>lt;sup>c</sup> Chi-square value \*P<0.05

 $<sup>^{\</sup>rm d}$  Slope of the concentration-mortality regression line  $\pm$  standard error.

highly rates insecticidal effect against the LGB adults. Falodun et al. (2009) exerted that *Pyrenacantha staudtii* L. (Icacinaceae) EO led to different toxicities (up to 80%) against the LGB adults. In the present study, 11 PEOs caused mortality from 25 to 100% on the LGB adults.

Saidj et al. (2008) determined the toxic effect of Thymus numidicus L. (Lamiaceae) EO against the LGB adults. In addition, Kucukaydin et al. (2020) detected that *Th. cariensis* and *Th. cilicicus* EOs led to toxicity from 18.2 to 100% after 96 h of the treatment and at different concentrations against the LGB adults. In the study, Th. sipyleus EO caused mortality from 31.6 to 100% at three doses after 96 h against the adults of the LGB. Two studies support each other. Shaaya et al. (1991) established the toxic effects of EOs from sage, bay laurel, rosemary and lavender plants against the LGB adults. In another study, it was assigned that O. onites and O. vulgare EOs led to mortality 31.6 to 83.3% after 24 h of the treatment and at different concentrations against Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) (Demirel & Erdogan, 2017). Kordali et al. (2008) investicated insecticidal activities of Turkish O. acutidens EO on Sitophilus granarius and Tribolium confusum (Coleoptera: Curculionidae and Tenebrionidae) adults. O. acutidens EO led to mortality from 68.3% to 36.7% on two pest adults after 96 h of exposure, respectively, in their study. In the present study, the EOs of O. vulgare, O. onites, O. syriacum, Th. spicata and Th. sipyleus plants caused between 95.0 and 100% mortalities of the LGB adults after 96 h of exposure (Table 2). In their study, Yildirim et al. (2011) reported that the mortality

percentages at 96 h of treatment with the 1, 5, 10 and 20 µLpetri<sup>-1</sup> of S. hortensis EO were 97.97, 100, 100 and 100% in S. granarius adults, respectively. In another study, it was showed that S. hortensis EO was toxic to Cydalima perspectalis Walker (Lepidoptera: Crambidae) (Gokturk et al., 2020). In our study, S. hortensis EO caused mortality 100% at all doses (5, 10 and 20 μLpetri<sup>-1</sup>) after 96 h on the LGB adults. Similarly, the mortalities after 96 h with the 20 µLpetri<sup>-1</sup> of S. spicigera and Th. sipyleus EOs were detected as 94.27 and 82.82 % for S. granarius, respectively (Yildirim et al., 2011). In this study, it was recorded that S. spicigera and Th. sipyleus EOs led to mortality 100% at 20 µLpetri<sup>-1</sup> after 96 h on the LGB adults. The findings of these two studies are parallel with our study results for sensibility of the LGB adults to EO isolated from S. hortensis (Table 2). Besides, all mentioned studies confirm results of our determination related to insecticidal activities of the tested 11 PEOs belonged to Lamiaceae family. Therefore, in our study it is recommended that, when using EOs as an insecticidal agent against the LGB, it is good to be applied at 5, 10 and 20 μLpetri<sup>-1</sup> and exposed for 96 h.

#### **CONCLUSION**

The results of our study suggested that the EOs of tested plant species could have different toxic effects and the mentioned dissimilarities are due to their peerless chemical composition. Satureja cilicica L., S. cuneifolia Ten., S. hortensis L., S. spicigera (C. Koch) Boiss., S. thymbra, S. montana L., Origanum vulgare, O. onites, O. syriacum L., Thymbra spicata L., and Thymus sipyleus Boiss.) (Lamiaceae) have possible for development as new powerful biocontrol factorson the LGB adults. The improwing of natural insecticides will help to deminish the negative effects (residual problems, toxicity to users, environmental pollution, resistance of chemicals etc.). So, biological insecticides may be also effective, selective, biodegredable and associated with low of the pest, being less toxic to environment and human health. Our results and other reported earlier studies demonstrate the possibility of using the tested EOs of 11 different plants as insecticides against the LGB adults. The effects of the tested EOs at low doses may reduce the residuel effects and less hazardous than others to humans and environment.

As the result of this study, it was suggested that the fumigant effectiveness of the PEOs in the study must be field-tested in the storage conditions and the tested EOs permit further researchs into their development for the control of wheat insect pests.

#### **ACKNOWLEDGEMENTS**

Authors thank Mohamed Said Omar (Ondokuz Mayıs University), translator and native English speaker, for his worthy contributions in revising the English of this article; and also Yahya Nas and Necat Togay (Mugla Sıtkı Kocman University) for their technical assistances, valuable advices and contributions in the study.

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#### **CHAPTER 12**

# THE EFFECTS OF HONEY BEES, WHICH PLAY A KEY ROLE IN ORGANIC FARMING, ON POLLINATION, YIELD AND QUALITY

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#### INTRODUCTION

The world population we live in is increasing rapidly every year. However, the needs of this population such as food, shelter and a healthy environment are increasing day by day, too. Among these needs, environmental health comes to the fore along with healthy and safe food. Especially, new production techniques have been developed to meet the food needs of this increasing population. Grain legumes, which have a very important place in a balanced and quality diet, consist of dry beans, peas, cowpeas, beans, lentils and chickpeas. One of the basic elements of a balanced diet is proteins, and it must be met from plant and animal foods in daily diets (Togay et al. 2021). In addition, excessive fertilization and irrigation, unconscious pesticide consumption, use of modern tools and machinery have increased rapidly in order to obtain more products from the existing agricultural areas. With modern (conventional) farming, the food problem of the increasing population has been solved to some extent. However, it has caused irreparable problems on human and environmental health. The main negative effects of modern farming (agriculture) are problems such as deterioration of public health, climate change, deterioration of natural balance, decrease in biodiversity and environmental pollution (O'Kane, 2012; Conway, 2012; Stoate et al., 2009). Especially, in modern farming, synthetic pesticides widely used in the control of plant diseases and pests have caused different problems such as environmental pollution due to their slow biodegradation. However, these chemicals can cause toxic residues in treated products (Barnard et al., 1997; Isman, 2000; Misra & Pavlostathis, 1997). In adition, the

chemicals used casually in conventionel farming has led to a decrease in biodiversity and pollinator insect populations in the nature. Biodiversity decline causes losses of ecosystem functions, such as biological pest control and insect pollination (Thompson et al., 2014). For these reasons, the concept of organic farming (agriculture) has come to the fore. Organic farming is generally considered a more ecological alternative to conventional farming. A serious alternative to conventional agriculture is organic farming, which prohibits the use of synthetic inputs (Wintermantel et al., 2018).

#### 1. ORGANIC FARMING

Organic farming is an eco-friendly agricultural production technique. It is a type of agriculture that used only cultural measures, biological control and organic-based inputs without using syntetic chemical inputs by choosing appropriate ecologies in order to make plant or animal production without disturbing the balance of the nature. In other words, organic farming is a form of controlled and certified agricultural production at every stage from production consumption, without using any chemical inputs in plant and animal production. It is a concept that emerged due to the negative effects of modern farming on human and environmental health. unconscious and uncontrolled use of synthetic chemicals. and faulty fertilization in inappropriate production methods conventional farming caused the deterioration of the natural balance. In addition, they led to the decreasing of biodiversity and the occurrence of environmental pollution. For these reasons, the concept of organic farming has gained great importance. Organic farming provides the best possible relationship between living organisms and agricultural soil (Nandan & Gami, 2015). Considering all these, the main purpose of the organic farming is to protect the environment, plant, animal and human health at the highest level without polluting the soil, water resources and air.

### 1.1 Benefits of Organic Farming

There are uncountable benefits of organic farming. Some of them;

- Organic farming increases bio-diversity, long term soil fertility and the quality of food.
- It provides sustainable agricultural production in the long term.
- It protects the physical, chemical and biological balance of the soil.
- It reduces erosion by keeping the soil moist.
- It prevents the pollution of ground and surface waters.
- It supports long-term control of pests and diseases by using natural enemies without harming the environment.
- It prevents environmental pollution due to not using synthetic chemicals.
- It protects natural balance and wild life.
- It ensures the compatibility of production in agricultural areas with the environment.
- It provides healthier and safer food for society.
- It contributes to the society being more educated and conscious.

- It provides employment opportunities to enterprisings and helps to reduce unemployment.
- It decreases rural migration from villages to cities.
- It encourages all people to consume organic products. From this perspective, the importance of organic agriculture is more increasing.

In addition to the above-mentioned, organic farming makes a great contribution to the producers' budget and the country's economy. It increases the producers' welfare level (Nandan & Gami, 2015).

Turkey, known as minor asia, thanks to its climatic conditions, topographic structure and geographical location, is home to many bee species as well as a wide variety of vegetation. Due to this geographical feature, very fertile agricultural areas have been formed in Turkey and many different plant species are grown here.

Christenhusz & Byng (2016) recorded that there are approximately 374,000 known at present and described plant species in the world. Among them, 295,383 plant species are flowering plants. However, the number of the species is increasing day by day. As a result of the studies carried out in recent years, it has been noted that over 200 thousand plant species attract many insect species both with their (flowering) appearance and with the semiochemical substances they secrete during their growth and development periods, and about 20.000 plant species of these are visited by bees (Metcalf, 1986; Metcalf & Lampman, 1989; Kesdek & Yıldırım, 2006; Güler, 2006). For these flowering plant species, first pollination and then

fertilization must occur in order to bear fruit and ensure the continuation of their generation. Because organic farming prohibits the use of synthetic chemicals, it is known for diversified crop production to improve bee populations. It would likely improve floral resources and provide more habitat for many different species of bees (Bicknell, 2018). For these reasons, bee species tend to organic farming areas, which are a healthy and natural environment. Among these bees, honey bees are of great importance. They provide pollination as they pass from flower to flower to collect nectar and pollens. Honey bees are probably the most important insect pollinator for especially organic farming in the world. When the bee is mentioned, the honey bee (Apis mellifera L.) comes to mind immediately. However, there are about 10 more honey bee (Apis L. genus) species other than A. mellifera. Honey bees constitute the most important pollinator insect group in the nature, as they are spread almost all over the world in order for people to obtain honey and other food products (propolis, royal jelly, beeswax, etc.) (Özbek, 2008; Çalmaşur & Özbek, 1999). During the migration of ancient people, honey bees migrated with them from country to country. However, Wintermantel et al. (2018) highlighted that despite the importance of honey bees as pollinators of crops and wild plants, the effects of organic farming on honey bees couldn't exactly understand to understand.

# 1.2. Systematics and Morphology of Honey Bees

Bees, which are insects that make up the Apiformes group belong to the superfamily Apoidea in the order Hymenoptera (Brothers, 1975). Among them, honey bees include in the *Apis* L. genus of the Apidae family. *Apis mellifera* L., known as main honey bee, is the most important pollinator insect in the agricultural and natural areas in not only Tukey but also in the world. There are 11 different honey bee species (Otis, 1997) and 27 honey bee races that have been identified all over the world. Five honey bee races (*Apis melifera caucasica*, *A. m. syriaca*, *A. m. anatoliaca*, *A. m. meda*, *A. m. carnica*) distributes in Turkey (Ruttner, 1988; Kekeçoğlu et al., 2021).

Kingdom: Animalia

Phylum: Arthropoda

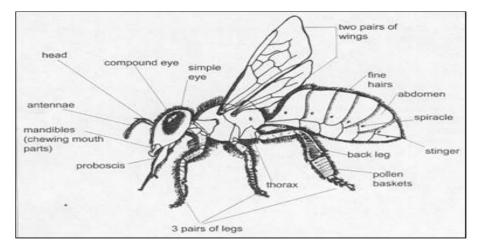
Class: Insecta

**Order:** Hymenoptera

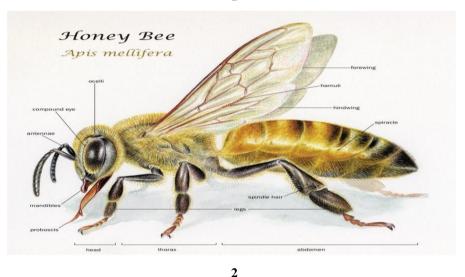
Family: Apidae

Genus: Apis L.

**Species:** A. mellifera L.



1



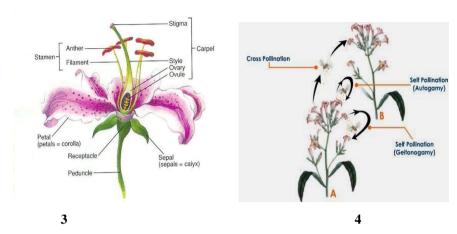
**Figures-1 and 2.** Morphology of the honey bee (https://www.google.com/search?q= Honey bee&tbm=isch&ved=)

#### 1.3. Pollination and Fertilization

The process of transporting the pollen produced in the male organs of flowering plants to the stigma of the same or different plant is called "pollination". It is an essential part of plant reproduction. Pollen from

the anthers of a flower (male part of the plant) is carried by a pollinator to the stigma (female part) of the same flower or another flower. The fertilized flower then produces fruit and seeds. There are many pollinator agents such as insects, humans, birds, and bats, water, wind. There are even self-pollinating plants with a closed flower structure without the need for a pollinator. "Fertilization" is the fusion of male and female gametes as a result of the pollination. In plants, fertilization is a process of sexual reproduction, which occurs after pollination and germination.

In some species of flowering plants, pollination is achieved by transporting pollen from the same flower or another flower on the same plant to the stigma by abiotic (wind, water) and biotic (insect, bat, bird, human, butterfly, moth, etc.) factors. Such plant species are called "self-pollinating" plants. In many plant species, the pollen must come from the flower of another plant of the same species, on the stigma of the flower of this plant, in order for the fruit and seed attachment to be as required. Pollination in these plants is called "foreign pollination" (cross polination) (Free, 1993; Çalmaşur & Özbek, 1999).



**Figure- 3.** The structure of a flower foreign

**Figure- 4.** The self-pollinating and pollination

(https://www.google.com/search?q=the+structure+of+the+flower)

(https://biology-igcse.weebly.com/-self-pollination-cross-pollination.html)

Plants in terms of pollination; It is divided into three as "monophilic" (pollinated by only one pollinator), "oligophilic" (pollinated by several pollinators) and "polyphilic" (pollinated by a large number of pollinators) plants. In terms of pollination, the pollinators are divided into three groups: "monotropic" (pollinating only one plant species), "oligotropic" (pollinating a few plant species) and "polytropic" (pollinating many plant species). All pollinators except honey bees take part in one of these groups. However, the honey bees vary from polytropic to monotropic behavior. However, plants that are pollinated by insects (bees, flies, butterflies, etc.) are called "entomophilous plants" (stone fruits, apple, figs, etc.) (Doğaroğlu, 1985; Çalmaşur & Özbek, 1999).

The role of bees in the pollination of flowering plants, especially entomophilous plants, is quite a lot. Especially, in apple, pear, peach, apricot, cherry, etc. such as fruit trees; in clover, sainfoin, clover, apricot, vetch etc. such as forage crops; in industrial plants such as sunflower, safflower, rapeseed, cotton, sugar beet; in the pollination of vegetables such as melon, watermelon, zucchini, many other cultivated plants and many wild plants, there is an absolutely need for bees. In fact, it is not possible for many plants to pollinate and bear fruit without bees. Especially in fruits such as apples, pears, almonds, apricots, and cherries, the effects of honey bees on pollination, yield and quality is quite considerable (Free, 1993; McGregor, 1976; Özbek, 1986; 1992; Çalmaşur & Özbek, 1999). Honey bee pollination significantly effects an increase in fruit yield, weight and quality. Honey bees are the most important insect pollinator in the agricultural and natural areas. But, honey bee populations are in decline day by day, due to different factors such as honey bee pests (Acarapis woodi Rennie, 1921; Varroa destructor Anderson & Trueman, 2000), different diseases (*Pseudomonas aeruginosa*, *Morator aitatulas*), poor diet, and synthetic chemicals. Therefore, honey bee pollination is critical to the production of organic farming crops in Turkey and the World. If there were no honey bees, the world would be a very different and uninhabitable place. Without they we would not have vegetables, fruit, seeds, nuts, and much more (https://www.lib.uidaho. edu/digital/objects/guidedreading/guidedread087.pdf).

# 1.4. The Effects of Honey Bees on Pollination, Yield and Quality of Fruits

In the light of the literature, we can list the effects of honey bees on pollination, yield and fruit quality as follows;

Khalifman (1959) recorded that plants pollinated by bees are less affected by late spring frosts than plants pollinated by other factors such as water, wind, birds, flies etc.. In the late 1800s, it was demonstrated that the pollination of fruit flowers was carried out by honey bees, their importance in pollination was well understood in the early 1900s, and it was observed that the yield increased significantly when beehives were placed in orchards (Menke, 1954; Özbek, 2008). Özbilgin (1999) reported that 70% of the plants in the world are pollinated by bees, and more than 80% of them are honey bees. It was stated that 90% of the pollination should be carried out by honey bees in order to obtain more and high quality fruit in strawberries (Fletcher, 1917; Skrebtsova, 1957; Mommers, 1961). In another study, it was determined that blackberry flowers produce plentiful amounts of nectar and pollen to attract honey bees, and as the number of visits by bees to the flowers increases, the size and amount of the fruits obtained increase accordingly (Shoemaker & Davis, 1966; Sherman & Westgate, 1968; Ağaoğlu, 1986). It was reported that raspberry flowers attract honey bees more because they produce a high amount of nectar, 90-95% of pollination is provided by bees, and the number of grains and fruit weights increase depending on the number of visits and the duration of the visit (Hansen & Osgood, 1983; Ağaoğlu, 1986;

Oliveira et al., 1991; Gianessi et al., 2002). Mel'nichenko (1977) stated that 50-60% yield increase in apple and pear and 25-30% yield increase in grapes can be achieved in pollination with honey bees. In a study conducted in Romania, fruit setting rate and product yield were 17% and 38 kg, respectively, when four hives of honey bees were placed per hectare in a cherry orchard, 14% and 32 kg when 2 hives were placed per hectare, and the hives were at a distance of 400 and 1000 m outside the garden While these values were 10% and 18 kg. 9% and 14 kg, respectively (Balana et al., 1983). A study carried out on on the avocado plant, it was counted an average of 788 fruits per tree when flowers pollinated by honey bees, and an average of 227 fruits per tree obtained from plants that were pollinated without honey bees (Vithanage, 1990). In many berry-like fruits (especially strawberries) and fruit types with a high number of seeds (kiwi etc.), the number of seeds of the fruits increases significantly as the visit of bees to the flowers increases, which allows the fruits to be of regular shape and to have a higher taste and aroma (Blanchet et al., 1991; Goodwin et al., 1991; Svensson, 1991).

Traynor (1999) reported that a total of one million bee colonies were rented in 1999 in California, which is among the most important almond production areas in the world, and they were used for pollination and yield increased. It was found that pollination is a very important factor on the yield of Blackcurrant, if this is achieved by honey bees, three times more yield is obtained and at the same time,

fruit size increases (Hofmann, 1995; Koltowski et al., 1999; Denisow, 2003).

Morse & Calderone (2000) stated that there had been an increase of approximately 10% in yield with the use of bees for pollination in apple orchards in the last 20 years in the USA. They recorded that this increase was due to the increase in the number of bee colonies used in pollination from 250,000 to 275,000. The same researchers attributed the lack of increase in cherry, sour cherry and pear production in the last 10 years to the fact that the use of bee colonies in pollination was not sufficiently increased. It is reported that the blueberry plant blooms quite a lot and has an important role in the pollination and yield of honey bees (Pavlis, 2004). Özbek (2008), in his determinations on a wild plum (Prunus sp.) species common in different localities of Eastern Anatolia, noted that more than 95% of the insects (bees) found in the trees around the beehives were honey bees. However, the number of honey bees was very low in places located 2000 m away from the beehives. In addition, the same researcher emphasized that 81-97% of the bees in a stone fruit tree and 45-95% in a pome fruit tree (apple) are composed of honey bees and they have great importance in the pollinations of these plants. In a study conducted in an apple orchard, the fruit bearing rate was determined as 33% on trees 10 m away from honey bee colonies. It was 18% in those 50 m away. The fruit bearing rate decreased as it gets further away from honey bee colonies (15% in 100 m). It was recorded that this value was 13% at 200 m and 9% at 300 m, and it

also affected the yield and quality (Mishra et al., 1976). Testolin et al (1991) investigated the effects of fruit setting by pollination of kiwi flowers by honey bees and wind. As a result of their study, they stated that the effect of wind on fruit setting was 68%, and the effect of bees was 98%. Howpage et al. (2001) investigated the effects of honey bees on pollination and fruit quality of kiwifruit in Australia. As a result of this study, they determined that bees pollinated 91% of the flowers and greatly affected fruit bearing, and this rate was 24% without bees. In a study conducted on the kiwi plant, it was determined that as a result of pollination by honey bees, there was a significant increase in fruit yield, quality and quantity, and the vitamin C content of the fruits was higher (Kuvancı et al., 2011). In the kiwi garden, it was found that 98.92% of the flowers blooming on the trees that were allowed to enter honey bees, and 32.08% of the flowers in the trees that were closed to the entrance of honey bees fructified (Kuvancı et al., 2013). It was determined that a honey bee makes 5-10 trips a day on different plant species, visits an average of 100 flowers in each of its wanderings (Morse & Calderone, 2000; Genç & Dodoloğlu, 2002). Considering this situation, it has been determined how important honey bees are in pollination in both self-pollinating and foreign-pollinated plant species. Crane (1975) recorded that the products obtained by pollination of bees worldwide is more than 50 times the value of honey produced that year. Levin (1983) emphasized that the economic value of the products obtained as a result of bee pollination in 1980 in the USA was approximately 143 times that of honey and beeswax in the same year. Robinson et al. (1989) emphasized that honey bees realized pollination 80% of flowering plants and contributed nine billion dollars per year to the agricultural production of the USA. Delaplane & Mayer (2000) stated that 90% of human food worldwide was obtained from 82 plant species and 63 (77%) of them were pollinated especially by honey bees. Winston & Scott (1984) reported that the product value obtained by bee pollination in Canada was 390 million dollars in that year. Matheson & Schrader (1987) emphasized that 2,553 million dollars were gained from pollination by honey bees in New Zealand in 1987. The same researchers stated that 797 million dollars of this income is obtained from vegetable and fruit products, and 10 million dollars from seed production.

In addition to all these, honey bees play an important role in the pollination of flowering plants growing wild in nature as well as in organic farming areas. As a result of pollination of wild plants, it helps to their reproduces by giving seeds, and these plants prevent the erosion known as the soil lost, especially in barren, windy and sloping lands. In addition, honey bees contribute greatly to the protection of natural life, the continuation and enrichment of biological diversity, and meeting their food, nest and shelter needs. Also, honey bees have a special importance in terms of meeting the various needs of humans (such as fuel, medicinal drugs, shelter, paint, etc.) from these newly formed plants as a result of pollinating wild plants.

#### **CONCLUSION and RECOMMENDATIONS**

Beekeeping and organic farming have become two important developing sectors in Turkey in recent years. Turkey takes an active role in beekeeping due to its peculiar ecology, rich flora and genetic variation in bee material at the present time. Thanks to these features, there are over 10,000 flowering plant species and various bee races and ecotypes adapted to regional conditions. Among them, honey bees are important in the pollination not only in Turkey but also in the world. While visiting from flower to flower, honey bees carry pollen taken from the anthers of flowers to the pistil of the same flower or another flower of the same species; and this activity is described as "pollination". Therefore, honey bees are of great importance in agricultural production, especially in organic agriculture.

Considering the above all informations, the importance of honey bees in agricultural production in developed countries has been better understood. It was emphasized that honey bees should be among the indispensable elements of especially organic agriculture. In addition to the contributions of honey bees to pollination and fertilization of flowering plants, it has been observed that they have an effect on not only the yield but also the quality of the seeds and fruits formed by the bees. These contributions of honey bees are invaluable, especially in organic agriculture, which is a healthy and safe form of food production. However, despite the fact that Turkey has a rich bee potential with its geographical location, vegetation and climatic conditions, it is a very poignant situation that it is not at the desired

level in terms of plant production and beekeeping. For these reasons, in addition to the correct use of other agricultural techniques, honey bees should be seen as an indispensable element of agriculture in organic farming areas in our country as well as in developed countries and used successfully in pollination. In this way, in addition to increasing the quality and quantity of plant production, it will enable beekeepers to be economically strong, and the yield of honey and other bee products, which are low per hive, will meet or exceed world standards. In addition, the fact that it is a source of employment for around 150,000 people, most of whom live in rural areas and do not have sufficient land, reveals the importance of beekeeping for our country's economy. Given all these positive contributions, it will also be very useful to raise the awareness of the producers about the importance of using honey bees in the pollination of plants grown in organic agriculture. In addition, giving "Pollination Credit" to especially of organic fruit growers in return for using honey bees will increase the use potential of honey bees in organic farming. However, the use of the synthetic chemicals should be strictly prohibited in the vicinities of organic agricultural production areas and serious inspections should be made. Also, to make the use of honey bees more widespread, the beekeepers should be supported financially with bank credites by the related ministry and encouraged to hire bee colonies. Similarly, making it compulsory to use honey bees for pollination by the relevant ministry in agricultural production enterprises will also increase yield and quality, and will also contribute to the development of beekeeping.

Finally, modern agriculture, which causes significant damage to human and environmental health, should be reduced and farmers should be encouraged to turn to organic agriculture. Also, supporting the use of honey bees, which have a great impact on pollination, fertilization, fruit yield and quality in organic agriculture, will ensure healthy and safe food production, and human and environmental health will be protected.

In this study, it has been given to effects on the pollination, fruit pruduction and quality of honey bees in organic and modern farming areas together with the literature.

#### ACKNOWLEDGEMENTS

Author thanks Necat Togay (Mugla Sitki Kocman University) for his technical assistance, valuable advices and contributions in the study.

In addition, a part of this study was presented as poster at the "5th International Muğla Beekeeping and Pine Honey (1-5 November 2016, Fethiye, Muğla, TURKEY)" Congress, and its summary was published in the Congress Book.

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# **CHAPTER 13**

# **ORGANIC VITICULTURE**

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#### INTRODUCTION

The rapid increase in the world population, the developments in the industry in parallel with this, the high prolificacy brought by conventional production ensured the survival of humanity. Since it is impossible to increase the existing agricultural areas to meet the food needs of the increasing population, various chemical inputs have also been used to increase the yield, which has been the beginning of the vicious circle. Although chemicals increase productivity, chemical wastes have entered the food chain in various ways and become a threat to living things. This situation has brought the problem of food safety to the agenda, especially in economically developed countries, in other words, in countries that do not have problems with the food supply. Conscious consumers with high economic income have started to demand products produced naturally without harmful chemicals. For this demand, with the initiative of some manufacturers in Europe, the transition from conventional production to a more natural production model was encouraged by reducing the use of chemical inputs. In this way, the terms organic, biological, or ecological agriculture have emerged. This initiative, which started with some training on "biodynamic agriculture" in Switzerland in the 1920s, as it was called at that time, continued with events held in England and Japan. However, the real big step started to become widespread with the establishment of IFOAM (International Federation of Organic Agriculture Movement) in 1972. According to IFOAM, organic agriculture's purpose, scope, and target are summarized. Organic

agriculture is a production system that maintains the health of soil, ecosystem, and people. The goal is to combine tradition, innovation, and science to benefit the environment we share and provide a good life for everyone involved in life with fair relations. Organic agriculture methods are applied in many countries within laws and rules. Most of the standards on organic agriculture are carried out by the umbrella organization. This movement, which started in 1972, has developed and grown day by day and has gained importance as a production model spread over 72.3 million hectares today.

#### 1. ORGANIC AGRICULTURE

Organic agriculture is defined as cultivating an environment where a perfect balance is established between climate, soil, plants, animals, and people (Vukosavljević et al., 2016). It was born as an alternative method to overcome the adverse effects of increasing chemical fertilizer and pesticide use in agricultural production on nature and human health and commercial problems (Çelikyürek and Karakuş, 2018). In order to correct the deteriorated ecological balance in organic agriculture, it is aimed primarily to reduce the factors that trigger this process and make natural production as much as possible in the continuation of the process. Of course, there are some limiting factors in this natural production process. For example, the plant's genetic potential to be grown and its resistance to negative stress factors affect the high yield in this system. Areas that have fertile soils and limit the development of diseases, pests, and weeds are the lucky areas to evaluate this potential correctly. Therefore, the abundance of such areas is seen as an excellent opportunity for organic agriculture. Taking this opportunity is also related to the agricultural economic policies of the countries, and as a result, there has been globalization in agricultural production in the world. Grain production in the northern regions and grapes, citrus fruits, and some other fruits in the Mediterranean regions, in countries with temperate and subtropical climates, while the production of spices, coffee, cocoa, and other tropical fruits came to the fore in tropical climates. This situation in terms of climate advantage has also made a tremendous economic contribution for the developing countries in the said belt (Vukosavljević et al., 2016).

What kind of system is organic agriculture?

Organic agriculture is a production system that aims to produce quality and sufficient quantities, promotes and improves biological vitality (microorganisms, flora, and fauna, plants and animals), increases soil fertility, preserves this environment in the long term, preserves animal and plant genetic diversity, and reduces all kinds of pollution and contains completely biodegradable products (Vukosavljević et al., 2016). It is not correct to perceive organic agriculture as a form of agriculture without fertilizers and pesticides. This mode of production is an agricultural system with its principles, every stage of which is controlled and certified within the framework of a specific regulation, from production to consumption.

According to the most recently reported 2019 data, organic farming is globally in 72.3 million hectares of land. When this total area is evaluated based on regions, it is seen that 35.9 million hectares, which corresponds to 49.7%, is in Oceania (Figure 1). The lowest rate is in Africa (2 million hectares), corresponding to approximately 2.8% of the total area.

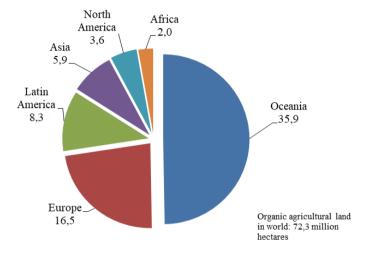


Figure 1. Organic agriculture areas (million hectares) by region (FiBL, 2021).

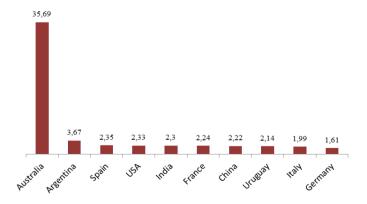


Figure 2. The ten countries with the largest organic area (million hectares) (FiBL, 2021).

Naturally, Australia (35.69 million hectares) is the most advantageous place in the world by organic cultivation. Afterward, there is a distribution as Argentina, Spain, USA, India, France, China, respectively (Figure 2).

India ranks first in terms of the number of organic producers. Afterward, Uganda, Ethiopia, Tanzania, Thailand, Peru, Turkey, Italia, Madagascar, and Togo are seen (Figure 3).

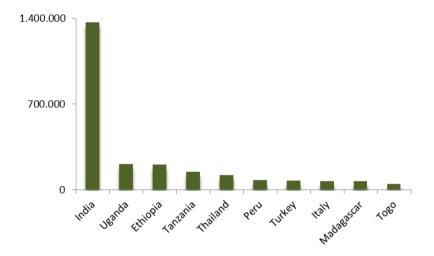
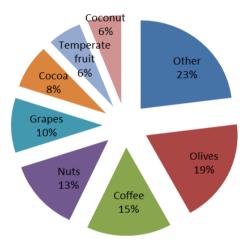


Figure 3. Top 10 countries with the most organic producers (FiBL, 2021).

Sales of organic products are growing steadily in industrialized countries. The positive view of the country's governments towards organic agriculture creates serious opportunities for the development of this production model. It is seen that developing countries follow the regulations of the countries that have a say in organic agriculture and make legal arrangements accordingly. Organic production is also an excellent opportunity for the agriculture of developing-

underdeveloped countries, which have advantages in terms of competition, especially with the advantage of the climate and product diversity that can be produced depending on it.

Olive ranks first among organically grown permanent crops in the world. Considering the climate selectivity of olive, which has critical importance in terms of its impact on human health and nutrition and its raw material for the agricultural industry, it is seen that subtropical climate regions are lucky regions for organic production.



**Figure 4.** Priority products in organic production in permanent crops (FiBL, 2021).

Among the permanenet crops that stand out in organic farming, olives are followed by coffee and nuts. Then comes the grape, which is cultivated in a wide area globally, has a numerous variety and consumption possibilities. Viticulture has a 10% share in permanent crops in organic farming (Figure 4).

# 2. ORGANIC VITICULTURE

Organic viticulture rearranges the ecological balance, which has been disturbed due to unconscious fertilization, irrigation, and mistakes made in combating agricultural pest and diseases, using the proper agricultural techniques. The aim is to use natural inputs to re-establish the disturbed balance, to make a natural feeding in all stages of fertilization, to use biological control in diseases and pests, as well as to use allowed compounds without toxic effects, residues, and thus to establish a sustainable viticulture cycle.

Success in organic viticulture is based on a production system that minimizes disease and pests and reduces the use of pesticides without sacrificing product yield (Sivcev et al., 2010). Here, an ecosystem creation approach in which each resource is optimized to sustain biodiversity, effective in reducing disease and pest pressure, is successful (Thies and Tscharntke, 1999).

The development and progress of organic viticulture in the world started in the 1950s and gained momentum in the 1990s. According to FiBL 2019 data, organic grapes are produced on an area of 467'760 ha globally, and this figure constitutes 5.3% of the world's total grape production area. Three hundred ninety-eight thousand six hundred fifty-nine hectares of this area are in the European continent. Certified organic vineyards in Europe are 381 thousand hectares as of 2019, which corresponds to approximately 84% of the world's total certified organic vineyard area. It is known that organic cultivation has shown a

rapid development not only in viticulture but also in all areas of agriculture, due to the support given to organic products and producers, information studies, legal protection, and planning activities in European countries (Özdemir et al., 2019).

While efforts to develop new varieties resistant to fungal diseases have gained importance in many European countries (Germany, Switzerland, Austria, Eastern Europe), this point of view has not yet become widespread in many other parts of the world. Due to the quarantine regulations in these countries, it is difficult to introduce new varieties, and due to this uncertainty, these varieties do not have a market yet (Geier et al., 2000).

Organic viticulture is common in European Mediterranean countries such as France, Spain, and Italy compared to other regions. The Mediterranean climate with hot and dry summers and a stable climate limits the development of pests and diseases in grapevines. In humid climates such as Romania, Germany, Hungary, Switzerland, and northern France, organic viticulture activities are mainly carried out with disease and pest resistant varieties, interspecies hybrids, and new resistant varieties (Vukosavljević et al., 2016).

North America follows the European continent quite far behind (27'444 ha). In third place is the continent of Asia with 17'141 ha. It is followed by Latin America (13'612 ha), Oceania (7'503 ha), and Africa (3'401 ha) (Figure 5) (FiBL, 2021).

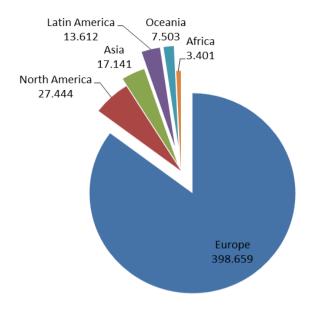


Figure 5. Organic vineyard areas by regions (kha) (FiBL, 2021).

In North America, the United States has the world's fourth largest organic vineyard area with 16'3 ha, according to 2019 data. About 60% of the organic vineyards are reserved for wine grapes, 30% for table grapes, and 10% for raisin varieties. The USA is increasingly turning to organic products in its consumption habits and is the world's largest market for organic agricultural products. However, increases in organic vineyard areas are slower than in other countries with similar areas. Due to the vineyards that are included in the organic system and come out of the system again, the size of the certified organic area fluctuates significantly (OIV, 2021).

3.3% of the world's organic vineyard areas are located in the continent of Asia. Turkey is the fifth largest producer in organic vineyard area with 15'1 ha (Table 1) in world, with an annual average growth rate of

9%. Turkey has been producing and exporting organic raisins since 1985 (Ateş et al., 2016) and is the world leader in organic raisin production. Organic grapes produced for drying are primarily concentrated in İzmir and Manisa regions, and almost all of them are exported abroad and primarily to European countries (Altındisli, 2004).

Organic vineyard areas in Argentina in South America are 4'4 ha (2% of the country's vineyard area) and tend to fluctuate constantly. It is currently the 10th in the world in terms of area and is the leader in region. 50% of the continental organic vineyard area is located in this country alone. Chile has 3'5 ha of organic vineyard area. Chile's main organic fruit product is wine grapes, and wine vineyards account for 45% of the total certified vineyard area (OIV, 2021).

While there has been an increase in organic viticulture in Oceania, there has been a severe decrease in the number of producers interested in organic viticulture. The share of organic vineyard area in the total vineyard area also decreased from 3% to 1.7% (OIV, 2021).

Organic vineyard areas in South Africa constitute 3.2% of the total vineyard area. This figure corresponds to 0.9% of the world's certified organic vineyard area. The rate of expansion of the certified organic vineyard area is relatively slow (OIV, 2021).

Organic grape production is carried out in a limited number of countries globally. By 2019, organic viticulture was carried out in 63 countries globally, and the certified vineyard area was 454 kha. This figure corresponds to approximately 6.2% of the total vineyard areas in the world. The largest organic vineyard areas are in Spain. Spain has 121'2 ha of organic vineyards. Spain is followed by France, Italy, the USA, and Turkey (FiBL, 2021) (Table 1).

Table 1. Top 10 countries by area under organic vines (FiBL & IFOAM, 2021)

Country	Organic area	Fully converted	Under conversion
	(ha)	(ha)	(ha)
Spain	121'279	99'386	21'893
France	112'057	68'506	43'551
Italy	109'423	83'825	25'599
USA	27'444	27'444	
Turkey	15'102	9'273	5'829
China	14'000	11'000	3'000
Germany	10'600		
Austria	6'567		
Australia	5'783		
Greece	5'488	4'283	1'204

Source: FiBL & IFOAM, 2021. Blank cells: Not data.

Although there are some changes in the regulations according to the countries, organic viticulture can be done in three different ways. First, it is possible to start with organic vine saplings at the stage of establishing the vineyard and continue this way. Secondly, the saplings used in the establishment phase are not organic, and production can be continued in organic viticulture after the conversion period right after planting. The third alternative is to produce organically after a 3-year conversion period in a vineyard that has reached the yielding period. All cultural transactions are planned, controlled, and recorded according to the relevant legislation. The conversion from conventional to organic production takes a certain amount of time. In this process, called the conversion process, the

necessary changes should be made according to a strictly determined plan. All standards in the conversion period have been clarified. Although this conversion period in the vineyard is usually three years depending on the control and certification institution, this period can be extended if necessary to form the standards fully. The harvest date is taken into account in the calculation of this period. Products can be marketed with the organic conversion label in this period. To be defined as an "organic product", it is necessary to obtain the organic product certificate by checking it at every stage by the control and certification institutions authorized by the relevant ministry following the regulation.

In the conversion to organic viticulture, there will usually be a decrease in production in the first year, especially in a vineyard that has reached the yield period. However, after the conversion period, the yield improves. Although most of the technical and cultural practices that should be done in conventional viticulture are similar to organic viticulture, organic viticulture has its privileges and parts that need attention.

# 2.1. Technical and Cultural Applications in Organic Viticulture

Treating the vineyard as an ecosystem is critical in optimizing cultural management methods. Cultural management methods are the most basic method in organic viticulture, as in other conventional viticulture, making the product less attractive to all kinds of pests. Here, the use of varieties resistant to fungi, especially at the initial establishment stage of the vineyard (Pedneault and Provost, 2016), is

of great importance considering the prevalence of fungal diseases like powdery mildew, downy mildew in the vineyards, and the reduction of yield experienced when not controlled. It is important to regulate irrigation and fertilization programs with effective sanitation such as removing harvest residues, pruning and similar wastes in vineyards (Mills and Daane, 2005; Jenkins and Isaacs, 2007; Zehnder et al., 2007).

### 2.1.1. Irrigation

First of all, the irrigation process to be carried out within a program prepared by considering the water consumption and need of the vine will increase the performance of the vine and indirectly will be effective in the combating pest and diseases (Daane and Williams, 2003; Stavrinides et al., 2010). In organic viticulture, industrial and city wastewater cannot be used for irrigation. The relevant institutions must give the certificate that the water is suitable for use in organic agriculture. A drainage system should be established in heavy soils with insufficient drainage, and drip irrigation should be preferred if possible. Irrigation, directly and indirectly affect insects and pathogens, which varies according to the irrigation method (drip or flood irrigation). If the irrigated plant is bustier and more attractive than the surrounding plants, the pest population may increase in such environments. Conversely, a drought-stressed plant may be attractive to insects, or the plant may not have the potential to tolerate the damage (Haldhar et al., 2017). It should also be noted that unnecessary irrigation will cause increased shoot density, shading,

reduce aeration, and encourage some diseases by preventing sunlight (Austin, 2010; Austin and Vilcox, 2011).

#### 2.1.2. Regulation of soil structure, plant nutrition, and fertilization

Although vines can grow at a slightly higher pH than many other cultivars, the soil must be in the appropriate pH range in terms of nutrient uptake. Micronized sulfur are used to lower the pH value in soils with high pH values. Humic acid is also used in organic viticulture to improve the physical properties of the soil and facilitate the uptake of plant nutrients.

Mineral fertilizers of natural origin are used in organic agriculture. The primary sources of mineral fertilizers are rock dust, lime, and rock phosphate, especially rich in calcium and potassium. Tree-plant ashes and bark, sawdust, seaweed, bird manure can be used. Liquid organic fertilizers are obtained from animal fertilizers or plant materials. These raw materials are left in the water for a few days or a few weeks; the organic materials are filtered and then diluted with clean water. The liquid organic fertilizers obtained are then applied by soil or foliar application. Commercial forms of some live microorganisms prepared for use in agricultural production, namely microbiological fertilizers, are also used. Plant growth-promoting bacteria and mycorrhizal fungi, which maintain a common life with the roots of plants, are also recommended for reasons such as improving the structure of the soil, facilitating the decomposition of organic matter, transforming mineral substances into forms that plants

can benefit from, binding atmospheric nitrogen to the soil, promoting phosphorus uptake and increasing stress tolerance. However, genetically modified microorganism preparations cannot be used. As green manures, mostly alfalfa, vetch, and broad beans, are planted, they increase the amount of nitrogen in the soil by taking the air's nitrogen into their bodies. Plants grown by planting between rows in autumn and winter should be mixed into the soil in the middle of the flowering stage.

The amount of farmyard manure used in organic agriculture varies according to the type and age of the animal, the type and amount of feed used, the housing conditions, and the animal's health. It also depends on the soil and climatic conditions and the amount of organic matter in the soil. After the leaves fall in the vineyards in autumn, it is necessary to spread the farmyard manures into the soil. The use of farmyard manure obtained from intensive animal production is prohibited.

# **2.1.3.** Tillage

Organic agriculture aims to maintain the soil's vitality and preserve its productivity. For this purpose, cover crop, mulching can be done. Processes such as tillage and the use of mulch contribute to the struggle by regulating the formation and diversity of natural enemies by supporting the activity of beneficial microorganisms (Powell et al., 2007; Sharley et al., 2008). In organic viticulture, as in other organic farming techniques, fewer processes are performed than the conventional method. Due to the soil structure and conditions in

organic agriculture, a large number of tillage is not done. Thus, it is advantageous in terms of energy and human labor. Soil cultivation methods should be applied to improve and protect the soil structure but not disturb the soil dynamics. However, these should not lead to water and wind erosion. It must be in a suitable form for microorganisms to live and prevent nutrient loss.

#### 2.1.4. Pest and Disease Management

Pest and disease management plays a key role in the conversion to organic viticulture as it can cause severe crop losses. Decreased product is seen as the most important problem for producers who plan to switch to organic viticulture (Merot et al., 2020).

Disease and pest control management is based on preventive measures rather than remedial measures in the organic farming system. This system gives priority to protecting the health of the ecosystem. Thus, the plant will become resistant to pests and diseases. Priority is given to the use of resistant varieties in organic farming. Plant breeders state that organic farming should focus on obtaining pest-resistant varieties. These varieties form a line of resistance with an effective defense. However, genetically modified plants cannot be included in organic farming. Some varieties may be less attractive to pest species or better tolerate damage. It is known that some plants rich in phenolic compounds, tannins, and flavonoids, as well as in appearance and color, have higher defense mechanisms (Haldhar et al., 2015). In addition, the actual product can be protected by using trap plants containing some compounds with attractive-repellent properties.

In organic farming, first of all, a preliminary defense against insects and diseases is created, and then the next line of defense is created with the use of ecologically safe plant chemicals and predatory insects (Haldhar et al., 2017). Except for the compounds allowed in organic viticulture, all synthetic inputs that leave residues in nature, have toxic effects on living things and disrupt the natural balance are prohibited.

# 2.1.4.1. Common vineyard diseases and their control in organic viticulture

Powdery mildew (Erysiphe necator Schwein) is the most common vine disease in the world. When this fungus is not combated with the appropriate control method, severe product losses are experienced. Cultural managements are the first and most important stage in the fight against this disease. First of all, the cultural measures mentioned above should be taken. Wettable sulfur and powder sulfur are used in the fight. Downy mildew (Plasmopara viticola Berl. & De Toni), another most common disease agent in vineyard areas is a fungus. When it is not combated, severe product losses are experienced, as in powdery mildew. Apart from cultural managements, producers can now receive all meteorological data with the early warning system and are informed about the most appropriate time to combating to diseases and pests. Based on the early warning system, it should be combated with copper preparations following the organic law. Gray mold (Botrytis cinerea Pers), also caused by a fungus, becomes a problem in rainy years and before the product is harvested on the vine. It is important to remove the pruning wastes and the clusters from the

previous year from the vineyard, provide ventilation and avoid excessive irrigation to avoid moisture. In Esca (Stereum hirsutum Pers., Phellinus igniarius Quél., Phaeoacremonium aleophilum W. Gams, Crous, M.J. Wingf. & Mugnai., Phaeomoniella chlamydospora Crous & W. Gams) disease is recommended to remove the diseased vines, pour quicklime into the empty pits, and leave it empty for a few years without replanting immediately. There is no struggle. In another disease, phomopsis cane and leaf spot (*Phomopsis viticola* Sacc.), cultural managements should be taken, especially the removal of pruning wastes from the vineyard, and copper-based pesticides should be used. In disease of armillaria root rot (Armillaria mellea) is recommended to establish vineyards away from forest trees, grow wheat and similar cereals for a while on lands that are cleared from forests, prevent rainwater from the forest from entering the vineyard, and remove pruning wastes (Özdemir et al., 2019).

# 2.1.4.2. Common vineyard pests and their control in organic viticulture

Grape bud mite (Eriophyes vitis) is common in vineyards. The pesticide allowed in organic viticulture is sulfur. In grapevine leafhopper (Erythroneura vitis), although it varies according to the licensing status of the countries, the pesticides allowed for use in organic viticulture should be used. In European grape moth (Lobesia botrana), preventing mating is an effective method used in organic viticulture. Using the warning system, it is decided to spray according to the first egg and first larvae. The pesticides allowed for use in organic viticulture should be used. Against to mealybug (*Planococcus citri*), ventilation must be provided very well. In winter pruning, peeling, and burning of the bark overwintered by the pest is recommended to reduce the density. In *Anaphothrips vitis*, care should be taken to ensure that there are no host plants around, and licensed pesticides allowed in organic farming should be used for spraying. In the fight to vine weevil (*Otiorhnychus spp.*, Megamecus shevketi), there should be no host plants around the vineyard. Siliconized fiber wrapping on the trunk and branches of vines is effective as a mechanical precaution in preventing adults from reaching and damaging the dormant buds. Vineyards should not be left grassy against to red spider mite (*Tetranychus urticae*), and predator insects are used in biological control. Summer oils and pesticides allowed for use in organic viticulture should be used for spraying (Özdemir et al., 2019).

# 2.1.5. Pruning and Training

There is no difference between organik and conventional production in terms of pruning and the way of training. The pruning needed by the variety should be applied. Pruning is critical because of its effect on reducing the severity of powdery mildew disease. In terms of good ventilation of the vine and preventing congestion, the creation of double T, Y, and V forms of training effectively reduces the need for spraying by preventing fungal infections.

#### 2.2. Organic Grape Products

Today, many products obtained from grapes can be produced and marketed organically. Table grapes, raisins, wine, molasses, and grape juice are the most common products (Özdemir et al., 2019).

#### 2.2.1. Organic table grapes

Organic farming practices in table grape production include cultivation practices. Only permitted additives may be used during the processing of the product. No chemicals should be used during the storage of organic grapes. Organic grapes should be stored separately from products grown using the conventional production method. In addition, care should be taken in selecting the product's packaging material. The organic product must be labeled.

# 2.2.2. Organic raisin

In organic raisins, the process including the cultivation and drying technique and the process during the storage of raisins are important. There should be no interference, contamination, or damage during harvest and transportation. If the drying process is to be carried out by immersion in the solution, the mixture (olive oil and potassium carbonate), the water used in the solution, and the mixing bowls must be usable in organic agriculture. The drying area must also meet the required criteria.

#### 2.2.3. Organic wine

The decisions of non-European organic wine producers regarding variety selection are often dependent on international market demands. Therefore, the main varieties grown today are Cabernet Sauvignon and Chardonnay. However, hybrids of older varieties are used in Canada and the United States. In Australia and California, mainly vinifera varieties are grown. Italian vinifera varieties are becoming increasingly important internationally (Geier et al., 2000). Wine is the product with the highest added value among grape products, but countries' perceptions of organic wine may also vary. While sulfite as a preservative in organic wine is not allowed in the USA, the situation is different in Europe. While the grapes used in wine production are organic, the wine processing and the additives used in the process must also be suitable for organic production.

# 2.2.4. Organic molasses

The materials used during the production of must, the clarification agents used in clarification, the calcium carbonate used to remove acidity, or the soil should all comply with the relevant legislation. Storage and packaging conditions must also meet the organic condition.

# 2.2.5. Organic grape juice

In the production of organic grape juice, in addition to being organic, all stages such as crushing, pressing, depectinization, clarification,

detartarization, filtration, and pasteurization must comply with the relevant legislation.

What information is on the label of the organic product?

On an organically produced and processed product, the name of the product, the year of manufacture, the name of the company to which it belongs, the name and logo of the certification institution, the certification number, the code number given by the committee, the product ingredient list, the expiry date, and the organic product logo must be present.

It should not be forgotten that a healthy diet without pesticides, antibiotics, heavy metals, and similar residues will also affect the health of future generations.

#### **Abbreviations**

IFOAM: International Federation of Organic Agriculture Movement OIV: Office International de la Vigne et du Vin (International Organisation of Vine and Wine)

FiBL: Forschung Institute für Biologischen Landbau (Research Institute of Organic Agriculture)

kha: thousands of hectares  $= 10^3$  hectares.

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ISBN: 978-625-8405-00-2