ADVANCE STUDIES IN SCIENCE

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CONTENTS

PREFACE

Distinguished Scientists,

We are pleased and happy to meet with many scientists who contributed to the scientific field and literature in this book. We would like to thank our valuable authors who have valuable studies in the book.

Over years, important advances have been achieved in the world to improve life quality and welfare of human. In recent years, there have been significant developments that hinder sustainable life and agriculture. In this regard, scientists and authors who writes chapters have valuable efforts on the issues. Humanity must do its best to protect the ecosystem from greater dangers. Small changes in the climate, can severely affect the life cycles of living organism and also this situation effects all productivity in scientific era. The main purpose of this book is not only talk about current issues in science but also suggest for solving problems for natural resources, nanotechnologies and life sciences which driven mainly by population growth, changing dietary patterns, industrial developments, urbanization, climate changes, chemical disaster.

In this book, there are 14 chapters which were prepared by authors. As editors, we would like to express our gratitude to all our authors who have made significant contributions with their knowledge, experience, and suggestions to our readers. We hope that this book will generate awareness towards new technologies in science, human benefits, and other living ecosystems along current and new developments in scientific studies.

Sincerely Yours,
November, 2022
Prof. Dr. Ahmet KAZANKAYA
Assist. Prof. Dr. Mevlüde Alev ATEŞ

CHAPTER 1

NON-CEREAL GRAIN

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INTRODUCTION

In recent years, the interest in Non-Cereal Grain (Pseudocereal) products has started to increase gradually (Yaver and Bilgiçli, 2020). It is known that these non-cereal grain plants were used as a food source in South America, Central Asia and Africa in ancient times. Although they belong to different families in taxonomic terms, they have similar purpose and nutritional content (Van Der Kamp et al., 2014). All cereals are monocotyledonous plants, pseudocereal, on the other hand, have a woody or herbaceous structure with an annual or perennial dicotyledonous. The proportion of starch in their content is very large in qualifying them as non-cereal grain (Ha-ger et al., 2012). Amaranth, Quinoa, Buckwheat and Tambourine plants have begun to gain importance as some of these pseudocereals. Amaranth and quinoa have an important place in the diet of ancient civilizations in the Andes, which are located in a wide geography such as Argentina, Peru and Colombia in the South American continent. Similarly, it has been revealed that buckwheat in the Asian continent and tambourine grain in the African continent were consumed in the oldest periods. Today, with the increase in their importance as an alternative food source, they have started to be referred to as the plants of the future.

Pseudograins are particularly noteworthy for their role in blends of gluten-free and functional foods production (Yaver and Bilgiçli, 2020). In the digestive system, various diseases occur due to the damage of hairless organelles in the intestines over time. One of these diseases is celiac disease which occurs due to gluten sensitivity. It is very important for this disease patients to consume gluten-free foods as an alternative to have a normal condition the rest of their lives. In addition, the use of pseudocereals as healthy nutrition and diet food in various ways has led to the emergence of a sector with high economic value. It has started to decorate various market shelves by producing many healthy products. This trend has started hundreds of scientific researches and started to be on the agenda in respected scientific journals.

Salinity and drought stress affects large agricultural lands in the world and in Turkey (Tan and Temel, 2019). Alternative crop products are essential to ensure sustainability in agricultural production, especially in food supply and security. In the world, plants that are resistant to water and salt stress (xero-halophyte) have become popular in human and animal nutrition, energy production, fiber industry, environmentally friendly landscapes, and in the

production of some chemical substances. Informing our society and to create an awareness by drawing attention to the importance of non-cereal grain plants with high original value, was the aim of this chapter.

2. QUINOA (*Chenopodium quinoa* Willd)

2.1. Situation in Turkey and in the World (Cultivation, Production Quantity)

It is cultivated in more than 50 countries, mostly in South America. World production in 2020 was 175 thousand tons of production harvested from nearly 189 thousand ha area in the world (FAO, 2022). Since it is a newly recognized planted crop in Turkey, reliable statistical data has not been recorded officially.

2.2. Botanical Features

Quinoa (*Chenopodium quinoa* Willd.) is a herbaceous plant belonging to the Amaranthaceae (Chenopodiaceae) family, like spinach and sugar beet (Picture 1). It is a species whose annual buds are protected within the seed (therophyte). Quinoa has a very strong, branched pile root system that can go down to 50-280 cm depth. Its tap root system provides an important advantage in drought resistance. Although the plant is tall, it varies between 50-350 cm. The stem has a spongy texture when looking at the inside cross-section. The plant has a hairless or slightly hairy leaf blade resembling a wide goose foot. The leaves can turn green, yellow, purple or red depending on the development period (Tan and Temel, 2019).

Although there are differences between regions, flowering occurs between July and August. The length of the panicle, which has a lot of branching in the form of a cluster, varies between 30-70 cm (Picture 2). The plant is 85-90% self-pollinating, bisexual (hermaphrodite), the flowers have an elongated anther and a female stamen with a 3-part stigma (Risi and Galwey, 1989).

Figure 1: Red population and Titicaca quinoa varieties (Photo: İ, BARAN, E. ORAL).

The achene-shaped fruits have a thin and delicate shell on them. The grains are 1-3 mm round shapes (Picture 3). Thousand grain weight of seeds varies between 1.99 -5.08 g (Reichert et al., 1986). It has a huge amount of color variation due to the saponin present in the grains. Because of these colors, it is used as an organic fabric dye in weaving.

Figure 2: Quinoa seeds (Web: https://www.vinmec.com/en/news/health news/nutrition /what-is-quinoa-and-can-it-be-eaten-instead-of-rice/)

Quinoa gives high yield in fertile deep-structured soils. Sowing date is between April-May using a seed rate 0.8-1.5 kg/ha. An average of 150 kg/ha of nitrogen fertilizer is required to fulfill the plant's nitrogen requirement, and in summer irrigation should be done once a week.

2.3. Importance and Use

Compared to other grains, Quinoa has a rich different seed structure in terms of bran ratio. It is an energy source with a very high ratio of fat and protein, rich in starch, and unsaturated fatty acids in a circle-shaped embryo. It also contains dietary fiber, vitamins, minerals, phytosterols and polyphenols (Taylor and Parker, 2002). It has been stated that quinoa products might prevent cancer by reducing the cholesterol level in plasma. Furthermore, it has been reported to have anti-inflammatory and antidiabetic effects and have a positive effect on hypertension (Verardo et al., 2018). It has been clinically determined that gluten-based nutrition has many negative effects on human health (Picture 4). The absence of gluten protein in quinoa grains emerges as an alternative food source with high nutritional value for celiac patients (Alvarez-Jubete et al., 2010). Although there is no gluten in rice, which is often consumed by celiac patients, the level of some nutritional elements are low, such as magnesium. Magnesium is makes bones stronger while balancing blood pressure and heart rhythm. When evaluated from this point of view, quinoa is a unique product as a source of calcium, iron, magnesium, zinc, manganese, potassium and phosphorus in terms of balanced nutrition of celiac patients (Mota et al., 2016).

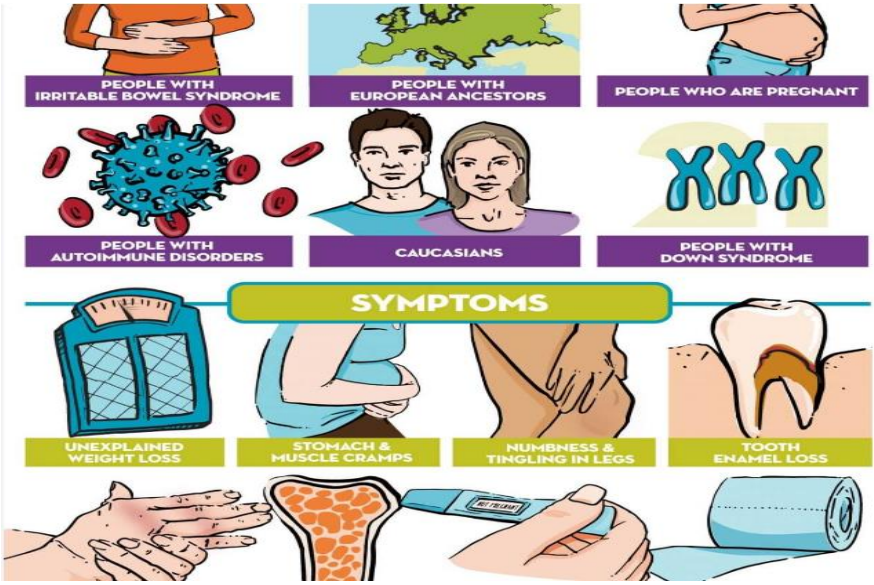

Figure 3: Symptoms of celiac disease (Web: https://share.baptisthealth.com /symptoms-celiac-disease/)

Research determined that the proteins found in the seeds of pseudocereal such as quinoa, amaranth and buckwheat are mostly composed of albumin and globulins. It has been proven that there is no storage protein called prolamin, which has a toxic effect for celiac patients, in pseudocereal (Drzewiecki et al., 2003). Unlike other cereals, the excess of lysine amino acids in pseudocereals such as quinoa draws attention. This amino acid has important roles in muscle building in the body. When all these research results are examined, it is seen how important pseudocereal are in terms of healthy nutrition (Drzewiecki et al., 2003).

3. AMARANT (*Amaranthus spp*)

3.1. Crop's World and Turkey Status (Cultivation, Production Amounts)

The Amaranth (*Amaranthus spp*) genus, whose originated is the Americas, includes about 60-70 plant species belonging to the Amaranthaceae family. It is stated that Amaranth was the main food source of the Aztec, Inca, Maya and American Indians. It is known that amaranth, is used in many religious and social rituals. Some of the species in this family are used as

vegetables, cereals and ornamental plants, and some are used as animal feed (O'Brien and Price, 2008; Anonymous, 2010; Lee, 2011). With the discovery of the new continent in the 16th century, the plant entered Europe as a cereal plant. It is produced commercially in India, China, Southeast Asia, Mexico, South America and the Andes Mountains, USA and Russia. In Europe, the Czech Republic is the country with the most amaranth cultivation with an area of 250 hectares, followed by Austria, Slovakia, Germany, Hungary, Poland, Russia, Italy and Slovenia (Belton and Taylor, 2002). Unfortunately, there is no official record of its production in Turkey.

3.2. Botanical Features

It contains a large amount of sub-varieties and it is possible to divide it into two grains and vegetables (Picture 6). Amarant, which is similar to quinoa in appearance, is a plant with a creeping or upright stem structure that can be up to 2 meters tall with or without hair (O'Brien and Price, 2008). Although there are many color variations in the flowers, stems and leaves, maroon or dark red are the most common colors. Grain amaranth plant has a hard, hairy, wide and colorful stem. Plant height can grow up to 150-210 cm in species produced as seeds. Amaranths have a long petiole arranged alternately on the stem. Leaves can be green, purple or red in color. The vast majority of amaranths are monoecious plants (O'Brien and Price, 2008). In plants that are monoecious, at first the flowers are green in the form of clusters at the tip of the shoot. There is a single male flower in the flowers. The colors of the flowers can be pink, white or purplish. The plant may have thorny or very stiff hairs on the plant. Amaranths also show a wide variation in seed color. Seeds are white or gray in those grown for grain, and black in varieties consumed as vegetables. There are approximately 1000-3000 seeds in 1 gram of amaranth (O'Brien and Price, 2008).

The crop should be sown at 1.5-2 cm depth and since the seeds are very small a very good soil preparation should be done. It is desirable that the soil temperature be 18-25 C. While 5-20 kg/da of nitrogen fertilizer is given with seeds per decare, it is planted so that 2 kg/ha of seeds in fall. Irrigation is done every 4-5 days according to the needs of the plant (Ergun et al., 2014).

Figure 4: Amarant species (Web: https://www.google.com/search?q=amaranthusg)

3.3. Importance and Use

It is very similar to quinoa in terms of herbal appearance. Amarant is used in the production of breakfast cereals, crackers, cookies, bread and other bakery products, especially in Europe and America. The green leaves of the amaranth plant can be collected fresh and used in different ways such as salad, boiled, steamed, fried or flavoring the food. Like all pseudocereals, amaranth seeds have a 6-10% oil content and have a lowering effect on cholesterol (Verardo et al., 2018). It can be used as an alternative food for those with gluten sensitivity such as oats. Amaranth contains a high percentage of mineral substances. It has been found to be very beneficial especially for celiac patients, who are known to be more prone to high calcium content, osteopenia and

osteoporosis . It is a source of vitamin E, which provides skin health, eye health and hormonal regulation in plant seeds (Taylor and Parker, 2002). The high content of manganese and zinc in the plant has an important place in the nutrition of children which can reduce autism (Fischer Walker et al., 2009).

4. BUCKWHEAT (*Fagopyrum ssp*)

4.1. Crop's World and Turkey Status (Cultivation, Production Amounts)

Central Asia is known as the gene center of buckwheat. Due to its origin here, it has spread to Russia and Europe via China and Japan. It was taken to the Americas in the 17th century through Europe and started to be cultivated (Jing et al., 2007). Another view on the distribution of this plant is that it originated in China and spread from the Greek region to cool and cold climate regions (Mazza, 1988). It is thought that the spread of this plant is due to its richness in nutrients, its rapid growth feature and its very comfortable cultivation in cold climates as well as infertile and fallow lands (Mazza, 1988).

Buckwheat production in the world was more than 1.8 million tons, more than 1 million tons was produced in China, and more than 0.7 million tons from Russia (FAO, 2022). Unfortunately, there is not enough production in our country to be reflected in the statistics.

4.2. Botanical Features

Buckwheat is a plant belonging to the Polygonaceae family. It is a fast-growing broadleaf annual plant sown in March-April. Plant height varies between 60-120 cm depending on the climatic and environmental conditions. The root system is single branched and branched around. The leaves are flat, and the triangular flowers are white, pink and red with fragrant makes it very important nectar source for honey bees (Picture 6).

Figure 5: Seeds, leaves and flowers of buckwheat (Web: https://kayseri.tarimorman.gov.tr)

The seeds of the plant are covered with a triangular fruit shell (pericarp). Shape, color and size vary according to varieties (Dizlek et al., 2009). The seed coat may be glossy black, gray or dull brown (Mazza, 1988).

4.3. Importance and Use

Buckwheat flour is used to make noodles in Asian and European countries (Skerritt, 1986). Buckwheat (*Fagopyrum esculentum* Moench) and Tatar buckwheat (Tartary Buckwheat-*Fagopyrum tataricum* Gaerth) are widely used in food production in the world (Choi et al., 2007). The grain contains protein 10.0-12.5%, starch 55-75%, fiber 7-10.7%, lipid 1.4-4.7%, ash 1.3-2.3% and moisture 9.6-13.8%. Furthermore, it contains high nutritional value protein and significant levels of dietary fiber, vitamins (vitamins B1, B2 and E) and minerals. Rutin and quercetin are the main antioxidants of buckwheat. They are considered in the treatment of chronic venous insufficiency disease. The main nutritional value of buckwheat grains is similar to that of cereals. Buckwheat, which has almost the same amount of starch and fiber as cereals, contains high levels of essential polyunsaturated fatty acids such as linoleic acid. Compared to cereals, buckwheat protein has a high nutritional quality due to its high concentration of all essential amino acids (especially lysine, threonine, tryptophan) and its balanced amino acid composition together with the sulfur-containing amino acids it contains. On the other hand, low digestibility has been noted due to tannins, phytic acid and protease inhibitors (Wei et al., 2008).

It has been found that a buckwheat-rich diet containing flavanols and their derivatives, along with other antioxidants such as tocopherol and phenolic

compounds, promotes the activity and growth of Bifidobacteria and Lactobacilli in the large intestine (Fesas et al., 2008). While buckwheat proteins are rich in albumin and globulin, they are poor in glutein and prolamin content. For this reason, gluten formation does not occur in dough prepared with buckwheat flour or cracked wheat flour. Buckwheat protein has high biological value due to its amino acid composition rich in lysine and arginine (Pomeranz and Robbins, 1972.).

It is stated that they cannot digest hydrothermally treated buckwheat starch in particular. Therefore, it is recommended to use buckwheat with foods with a low glycemic index. Foods with a low glycemic index play an important role in controlling diabetes and regulating the potential to raise blood sugar (Wijngaard and Arendt, 2006). When compared with white bread made with wheat flour with a glycemic index value of 100, it was determined that bread made with boiled buckwheat germ had a glycemic index value of 61.2, while buckwheat bread made with 50% raw buckwheat germ had a glycemic index value of 66.2 (Skarbanja et al., 2001). In this sense, the high amount of resistant starch in buckwheat; Nutrition in the axis of digestibility undertakes an important mission in terms of health in the axis of keeping blood sugar under control. In patients with celiac disease, the incidence of allergic reactions caused by buckwheat was determined to be 1% (Wieslander et al., 2001).

CONCLUSION

The importance of the crops in terms of and nutritive values and production has been documented. These features can be turned into an important advantage in the development of new foods and products. The benefits of these Non-Cereal Grain products for human health have been revealed by many existing studies. Especially bread and pasta consumed in daily life. It is seen that it can be used successfully in the development of the formulation of foods such as cakes, biscuits, tarhana and extruded products. According to sensory analyzes such as taste, smell and color, it has been understood that quinoa, amaranth and buckwheat can be used for bread production up to an average of 25%. It has been determined that it can be used up to 20% on average as pasta. The low nutritional quality in terms of protein, micro-nutrients and dietary fiber and their high carbohydrate content increase the importance of Non-Cereal Grain products in terms of gluten-free foods for

celiac patients, whose numbers have increased in the society in recent years. It is possible to produce products such as gluten-free bread, pasta and biscuits that are enriched with protein, oil, mineral substances and dietary fiber and that can be accepted as sensory. It is thought that the nutritional and functional properties of the products obtained by including amaranth, quinoa and buckwheat whole flours in food formulations will increase significantly and will be a good alternative for consumers.

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CHAPTER 2

THE EVALUATION OF IMPACTS OF ROW SPACINGS AND SEEDING RATES ON YIELD COMPONENTS IN RYE

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1-INTRODUCTION

Rye grown in the world and in Turkey is used directly and indirectly in human nutrition (Yanbeyi and Sezer, 2006). Germany and Northern European countries directly constitute the raw material of bread. It is indirectly used as a raw material in silage, green grass, hay and crushing (grinding grains) in animal nutrition and industrial industry (Yılmaz et al., 1996). The use of rye as a raw material for bread is increasing with the sensitivity of conscious eating habits developing in the world and in Turkey. Therefore, this provides an increase in the number of studies on the bread quality of rye. In the study conducted to determine the quality of rye bread made in Turkey, it was determined that the mixture of wheat flour with 30% rye flour gave the best results and it is recommended to make the bread in this direction (Mankan, 2008).

The world rye cultivation area is 4.4 million hectares and the production amount is 13 million tons. Most of the rye cultivation area and production is produced by Germany, Russia, Poland, Belarus, Denmark, Spain, Turkey, Canada, China, Ukraine and the USA (FAO, 2018). The rye cultivation area is 101.000 hectares, the production amount is 320.000 tons, while the kilogram yield is 316 kg per hectare in Turkey, in 2017 (FAO, 2018).

Rye has a high adaptability to adverse conditions. With its strong root system, it benefits very well from the nutrients and water in the soil. It is grown in unproductive soils with resistance to cold and hot climates, being able to grow in areas with 150 mm of precipitation, rough, stony and poor soil organic matter (Öztürkci, 2009).

Plant genetics, environmental conditions and breeding techniques are the most important factors determining yield and quality in plant production. Since it will be very difficult to make changes in environmental conditions in rye as in all plants, it is aimed to increase grain yield and quality per unit area with changes and cultivation techniques. It has been revealed in many studies that the most appropriate row spacing and seeding rates are important agricultural practices in grain yield increases. It has been reported in studies that factors such as the number of fertile spike per unit area, the number of grains per spike and the grain yield per spike are values that vary according to the plant density and directly affect the yield (Kaydan et al., 2011). The fact that the row spacing is narrower than 19.2 cm has an important potential to increase grain yield. But

they reported that this potential may vary depending on the variety and environmental conditions (Marshall and Ohm, 1987).

They reported that the number of fertile spike per square meter increased with the sowing rates, while the number of grains per spike, thousand grain weight and grain yield per spike decreased (Kaydan and Yağmur 2008). Even as the row spacing widens; flag leaf width, the number of spikelets per spike, the number of grains per spike were increased, while plant height, grain yield and harvest index decreased (Kaydan and Yağmur, 2008). In another study, Öztürkci (2009) reported that with the increase in seeding rates an increase in the number of fertile spike per square meter, the width of the flag leaf, the number of spikelets per spike, the number of grains per spike, the grain yield per spike, the grain yield, the harvest index and the decrease in thousand grain weight.

It is very important to eliminate this negative situation that may occur in yield components and to determine the most suitable row spacing and seeding rates for the ecological region where production is made. Therefore, in the study, the effects of five different row spacing (15, 17.5, 20, 22.5 and 25 cm) and four different seeding rates (300, 400, 500 and 600 seeds m^{-2}) on the grain yield (kg da^{-1}) and yield components in rye (*Secale cereale* L. var. Aslım-95) under semi arid conditions of city of Kırşehir in Turkey were investigated.

2-MATERIAL AND METHOD

Material

Research Location: The research was grown in Ahi Evran University Agricultural Research Application Experimental field in 2016-2017. The trial area in Kırşehir and has an altitude of 1107 m, latitude 39º9' north, longitude 34º10' east.

Climate Characteristics of the Research Site

The monthly total rainfall and monthly average temperature characteristics of the experimental site where the research was conducted are given in Table 1. The experimen setup was completed in mid-October 2016. All observations were completed in July 2017. It is seen in Table 1 that the temperature averages with similar values compared to the long-term averages in 2016 are observed. The first precipitation was received in November. In

addition, it has been determined that the total amount of precipitation is 92.4 mm less than the precipitation statistics for LTA. In other words, the 2016-2017 grain production season can be evaluated as a dry season. It has been determined that relative humidity has a very low value in 2016-2017 compared to the LTA, depending on the rainfall received.

Table 1. Climate values of 2016-2017 year and the long-term average (LTA*)

	Rainfall (mm)		Temperature (c)		Relative Humidity (%)	
Months	LTA	2016-2017	LTA	2016-2017	LTA	2016-2017
September	12.3	42	17.9	18.0	51.8	48.2
October	29.2	0	12.2	13.3	62.4	49.9
November	36.5	24.9	6.1	5.5	71.5	56.7
December	46.9	42.7	1.9	-1.3	77.8	77.3
January	45.4	28.8	-0.1	-2.4	78.6	77.9
February	35.2	4.9	1.3	1	74.6	67.0
March	37.5	41.5	5.5	7.3	67.6	60.8
April	45.3	29.0	10.7	10.7	63.6	52.4
May	43.3	49.9	15.1	15.2	59.9	59.5
June	36.2	18.4	19.3	20.7	53.5	54.3
July	7.1	0.4	22.8	26	47.3	36.0
Total	374.9	282.5				
Average			10.25	10.36	64.42	58.18

*LTA (lomg term Average, 1954-2015)

Soil Properties of the Research Site

Soil samples were taken from two different depths (0-30 cm and 30-60 cm). The results are given in Table 2. When Table 2 is examined, it is seen that it is weak in terms of organic matter according to the results of soil analysis. It is understood that it is rich in terms of potassium, phosphorus and calcium. The experimental area has a slightly alkaline and clay loam soil structure.

The chemical and physical properties of the soil were interpreted according to Kaçar (1995).

Table 2. Physical and chemical properties of field soil

Physical and chemical properties	Soil depth	
	0-30 cm	30-60 cm
pH	7.59	7.63
Salt %	0.02	0.02
EC (mmhos/cm)	0.52	0.56
Organic matter %	1.81	1.64
Phosphorus ((P2O5) kg da^{-1})	2.14	2.29
Potasium (K2O (kg da^{-1}))	66.62	51.47
Lime % (CaCO3)	27.9	28.39

Methods

The experiment was conducted in a split plot design with five row spacing and four seeding rates with three replications. The experimental design consisted of 20 split plots for a total of 60 plots. Row spacing (RS), (15, 17.5, 20, 22.5 and 25 cm) to the main plots and seed seeding rates (SR) to the sub-plots (300, 400, 500, 600 seeds m^{-2}).) has been determined. The row lengths of each plot of the experiment established in the research were planned to be 5 m and 6 rows. The plot sizes were determined as 4.5 m^{-2}, 5.25 m^{-2}, 6.0 m^{-2}, 6.75 m^{-2} and 7.5 m^{-2} according to the row spacing and seeding ratess used.

The experiment was established on dry conditions and fallow land and was carried out in accordance with the cultivation techniques suitable for the region. Considering the results of soil analysis, all of the phosphorus and some of the nitrogen were applied with planting date, and all the remaining nitrogen was applied in the spring during the after tillering stage. DAP (Di Ammonium Phosphate) fertilizer with the calculation of 15.0 kg da^{-1} was applied together with the soil and mixed with the soil, considering the nutrient elements of the soil of the trial area in the soil, and 5.0 kg da^{-1} of pure nitrogen (in the form of ammonium nitrate) was applied during the after tillering stage. Weed control was done manually before tillering after tillering and repeated as necessary.

Harvesting was done by hand on July 12 with the help of a sickle. After leaving 0.5 m from the plot corner and one row from the edges, the remaining plants were harvested by cutting with sickle in the plot area (four rows in the

middle). The plots cut with the help of sickle were put into pre-prepared labeled bags and made ready for threshing. Then, necessary measurements were made and blended in the laboratory.

Statistical analyzes

The variance analysis of the obtained data was performed using the MSTAT-C package program according to the "split plot in random blocks experimental design. Means were grouped with the “Duncan Multiple Comparison Test”.

3-RESULTS AND DISCUSSION

In the study, the effects of row spacing and seeding rates on a total of 6 characters were investigated. The characteristics examined in the study are 6 characters: number of fertile spike per square meter, plant height (cm), spike length (cm), number of grains per spike, grain weight per spike (g) and grain yield (kg da^{-1}).

Number of Fertile Spike

In this study, which was carried out by applying five different row spacings and four different seeding rates on the number of fertile spike per square meter.

Table 3. Variance Analysis Summary of the Study

		F Value					
Source of Variation	SD	Number of fertile spike	Plant height	Spike length	Num. of grains per spike	Grain weigh per spike	Grain yield
Replication	2	3.1736	0.0009	6.3906*	1.9574	1.7722	0.0333
Row spacing (RS)	4	5.2886*	3.5685*	0.4146	4.3768*	0.8732	5.4828*
Seeding rates (SR)	3	20.80**	6.90**	4.318*	7.535**	3.5554*	24.48**
RSxSR	12	3.04**	0.27	0.8238	0.9036	0.9261	2.0915*
General	59						
Coefficient (%):	-	9.50	5.91	6.37	8.78	10.48	10.06

* P≤0.05, P ** p≤0.01

It was found that the effect of row spacing on the number of fertile spike per square meter was found to be significant at the $p<0.05$ level. Furthermore the effect of seeding rates and the interaction of row spacing X seeding rates on the number of fertile spike per square meter was found to be significant at the $p<0.01$ level. .

The highest number of fertile spikes per square meter was 386.9 numbers of square meter from 15 cm row spacing. The lowest number of fertile spike per square meter was found 320.1 spikes m^{-2} from 25 cm rows. It is seen that the effect of row spacing on the number of fertile spikes per square meter was significant and as the row spacing increases, the number of fertile spike per square meter decreased. The highest number of fertile ears per square meter was obtained from the narrowest row spacing of 15 cm, while the lowest number of fertile ears per square meter was obtained from the widest 25 cm row spacing. Due to the increase in row spacing, there were significant decreases in the number of spikes per m^{-2}. Gençtan and Balkan (2008) reported that there was a significant decrease in the number of spike per m^{-2} due to the increase in row spacing. Also, similar results were determined in the findings of researchers such as Lafond, (1994); McLeod et al., (1996); Chen et al., (2008); Yağmur and Kaydan, (2008); Öztürkci, (2009).

Table 4. The number of fertile spikes per square meter at different row spacing and seeding rates

	Seeding Rates (seed m^{-2})				
Row Spacing (cm)	300	400	500	600	Avareage
15 cm	362.0 b-e*	356.3 cde	376.0 bcd	453.4 a	386.9 a
17.5 cm	304.5 efg	318.8 d-g	363.5 b-e	419.6 ab	351.6 ab
20 cm	233.3 h	342.5 c-g	395.0 bc	386.6 bc	339.3 b
22.5 cm	286.3 fgh	345.5 c-g	347.0 c-f	317.4 defg	324.1 b
25 cm	282.6 gh	331.3 c-g	322.0 d-g	344.6 c-g	320.1 b
Avareage	293.7 a	338.9 b	360.7 bc	384.3 c	

*The difference between the means with the same letter is insignificant (Duncan, $p\leq0.05$)

Table 4 are evaluated in terms of the effect of seedind rates on the number of fertile spike per square meter; the highest number of fertile spike per square meter was obtained at 384.3 spikes m^{-2} from 600 seeds m^{-2}. The lowest number of fertile spike per square meter was determined as 293.7 spike m^{-2} from 300 seeds m^{-2} seeding rates. It is seen that the number of fertile spike per m^2 increases with the increase of the seeding rates. Carr et al. (2003), Joseph et al. (1985), Holen et al. (2001) reported in their study that the number of fertile spike per m^2 was increased with the increase in the seeding rates. According to Atak and Çiftçi, (2005); Yağmur and Kaydan, (2008) the increase in competition between plants reduces tillering, they stated that there is an increase in the number of fertile spike per square meter due to the high number of plants per unit area.

The effect of row spacing X seeding rates interaction on the number of fertile spikes per square meter were significant at the $p<0.01$ level. The highest number of fertile ears per square meter was obtained 453.4 spike m^{-2} from the plots planted with 600 seeds m^{-2} and 15 cm row spacing. The lowest number of fertile spike per square meter was found to be 233.3 spike m^{-2} in the rates of 300 seeds m^{-2} in 20 cm row spacing. In this study, the number of fertile spike increased as the row spacing narrowed and the increase in the rates of seeds increased. This situation reveals that it increases the plant living area per seed. Similarly, Tompkins et al., (1991) reported that the highest number of fertile spike per square meter was obtained in narrow row spacing and high seed rates. Johnson et al., (1988) reported that the number of ears per square meter was the most affected character by the change in row spacing and planting density. It is thought that there is a decrease in the number of ears per square meter due to the fact that the plants grown in arid conditions are lost more quickly with the widening of the row spacing and they cannot get enough nutrients from the soil.

Plant Height

In this study, the results of the variance analysis performed on the plant height are given in Table 3, while the average values of different row spacings and seeding rates regarding plant height and the difference groupings of the averages are shown in Table 5. Table 5 are evaluated in terms of the effect of row spacing on plant height; The highest plant height was obtained in 25 cm

row space with 143.60 cm. The lowest plant height was 138.61 cm in 15 cm row space.

In this study, the results of the variance analysis on the plant height data are given in Table 3. The average values of different row spacings and seeding rates regarding plant height and the difference groupings of the averages are shown in Table 5. The highest plant height was obtained 143.60 cm from 25 cm row spacing. The lowest value of plant height was found 138.61 cm from 15 cm row space.

It was determined that the average plant height of the change in row spacing was between 138.61 and 143.60 cm. The highest plant height value was obtained from the largest row spacing. In the study, it was determined that the plant height increased as the row spacing increased. Öztürkci, (2009) determined in his study that the average plant heights are between 121.53 and 126.80 cm according to row spacing. He reported that the highest plant height was obtained from the lowest row spacing, and contrary to our study, plant height shortened as row spacing increased. In the study of Ulukan and Kün, (2007) in which they investigated the effects of row spacing (5, 10, 17.5 cm) on the yield of wheat cultivars in Ankara conditions, our findings do not agree with the results of plant length shortening as row spacing widens in all cultivars. However, in the study, it causes narrowing on the row in wide row spacing. Therefore, competition among plants increases. This situation is thought to cause an increase in plant height. It was also stated by Yanbeyi and Sezer, (2006) that plant height is highly affected by the cultivation technique and ecological conditions, as well as the genotypic character.

Table 5. The number of plant height at different row spacings and seeding rates

	Seeding Rates (seed m^{-2})				
Row Spacing (cm)	300	400	500	600	Average
15 cm	134.26	140.33	139.13	140.73	138.61 b
17.5 cm	137.93	140.60	141.56	145.86	141.49 ab
20 cm	139.66	141.06	141.46	145.86	142.01 ab
22.5 cm	138.13	141.66	144.66	146.60	142.76 a
25 cm	141.46	143.60	142.80	146.53	143.60 a
Average	138.29 b*	141.45 ab	141.92 a	145.12 a	

*The difference between the means with the same letter is insignificant (Duncan, $p \leq 0.05$)

When the averages given in Table 5 are evaluated in terms of the effect of seeding rates on plant height; the longest plant height was 145.12 cm from 600 seeds m^{-2} seeding rates. The shortest plant height was determined as 138.29 cm from the seeding rate of 300 seeds m^{-2}. In the study, it was determined that the effects of seeding rates on plant heights are important and the average plant height varies between 138.29-145.12 cm according to the seeding rates. Şehitoğlu (2007), in his study in which the effect of seeding rates on barley yield was determined, revealed that plant heights did not show a significant change according to seed rates and plant height levels were close to each other at different plant densities, while Torofder and Hossain, (1991); Bilgin, (1997); Ozturk et al., (2006) reported that as the seeding rates increased, the plant height also increased. According to the report of the researchers, they revealed that as a result of the leaves shading each other due to the increasing seeding rates, the plants extend their height more to reach the light they need. It is thought that the reasons for the similarities and differences in plant height are due to the cultivars, growing environments and cultural processes applied.

Spike Length

In the study carried out by applying different row spacing and seed rates, the data on the spike length are shown in Table 6. When Table 3, in which the results of variance analysis of the spike length are given, is examined, it was found that the effect of seeding rates on spike length was significant at $p<0.05$, while the interactions of row spacing X seed rate were found to be insignificant.

The averages of spike length in Table 6 are evaluated in terms of the effect of row spacing. It was determined that the average spike lengths depending on the row spacing varied between 10.45-10.84 cm. Spike lengths in 15 cm row spacing was 10.84 cm. Ulukan and Kün, (2007) reported that the change in row spacing did not affect the spike length according to the results of their study in the row spacing of different wheat varieties (5, 10, 17.5 cm) in Ankara conditions. In addition, the findings of Öztürkci, (2009) in his study using Aslım 95 rye variety, which reported that the spike length was between 8.8 and 9.2 cm and that the change in row spacing did not affect the spike length, supports our results.

Table 6. The number of spike length at different row spacings and seeding rates

	Seeding Rates (seed m^{-2})				
Row Spacing	300	400	500	600	Average
15 cm	11.78	11.00	10.01	10.58	10.84
17.5 cm	11.00	10.68	10.98	10.08	10.68
20 cm	11.00	10.95	10.13	9.98	10.51
22.5 cm	10.93	10.53	10.13	10.23	10.45
25 cm	10.63	10.51	10.43	10.63	10.55
Average	11.07 a*	10.74 ab	10.34 b	10.30b	

*The difference between the means with the same letter is insignificant (Duncan, $p \leq 0.05$)

The averages in Table 6 are evaluated in terms of the effect of seeding rates on spike length; the highest spike length was obtained with 11.07 cm at a seeding rates of 300 seeds m^{-2}. A proportional decrease in the length of the spike is observed with the increase in the seeding rates per meter square. In the study, it was determined that the average spike lengths were between 11.07-10.30 cm. It was determined that as the seeding rates increased, the length of the spike shortened. Although the spike length generally depends on the genetic structure of the plant, it is also affected by environmental conditions. Our findings were reported by Atak and Çiftçi, (2005); Süzer and Demir, (2012); Yağmur et al., (2021) were found to be similar to the findings in their studies.

Number of Grains per Spike

The variance analysis results regarding the number of grains per spike are given in Table 3, and the averages of row spacing and seeding rates and the difference groupings of the averages are given in Table 7.

The effect of row spacing on the number of grains per spike was significant at the $p<0.05$ level, while the effect of seeding rates on the number of grains per spike was found to be significant at the $p<0.01$ level. The interaction of row spacing X seeding rates was determined to be insignificant.

Table 7. Number of grains per spike at different row spacings and seeding rates

	Seeding Rates (seed m^{-2})				
Row spacing	300	400	500	600	Avareage
15 cm	52.1	47.9	49.5	44.8	48.6 a
17.5 cm	52.5	45.8	47.1	40.0	46.4 a
20 cm	51.3	43.0	44.5	42.3	45.3 ab
22.5 cm	49.3	49.2	44.6	46.9	47.5 a
25 cm	42.2	41.5	40.5	39.0	40.8 b
Avareage	49.5 a*	45.5 b	45.2 b	42.6 b	

*The difference between the means with the same letter is insignificant (Duncan, $p \leq 0.05$)

The highest number of grains in spike was determined with 48.6 from 15 cm row spacing, while the lowest number of grains in spike (42.6) was found in the widest row spacing (25 cm). Önmez, (1994) reported that the number of grains per spike decreased as the row spacing increased in Konya Karapınar's arid conditions on two different rye lines and supports our study. Also, unlike our study, Tompkins et al. (1991), Ulukan and Kün, (2007) in their studies on wheat and Öztürkci, (2009) on rye reported that the number of grains per spike increased as the row spacing widened.

The highest number of seeds per spike was determined as 49.5 from 300 seeds m^{-2} m^{-2} seeding rates. The lowest number of grains per spike was determined as 42.6 with 600 seeds m^{-2} seeding rates (Table 7). The highest value of the number of grains per spike was obtained from the lowest seeding rates (300 seeds m^{-2}). Kaydan and Geçit, (2005); Öztürk et al., (2006); Gençtan and Balkan, (2008); Kaydan and Yağmur, (2008); Öztürkci (2009); Dinç, (2010); Süzer and Demir, (2012); İpek, (2016) support the this research results

Grain Weight per Spike

Although the effect of row spacing on grain weight per spike was not statistically significant. Grain weight was determined as 1.103 (g) in the 15 cm row spacing, which is the narrowest row spacing. Although the row spacing is not important in terms of grain weight per spike, when the averages of the row

spacing are evaluated, it is seen that the grain weight per spike decreases as the row spacing widens. However, when Gençtan and Balkan, (2008) reported that experiment of the spacing between rows applied in the their experiment; grain weight per spike increased in the first year by increasing the spacing between rows. It is reported to decrease in the second year. Increasing the row spacing of the plants increases the rate of benefiting from sunlight, leading to a prolongation of the life span of the leaves (the duration of their green stay). With the prolongation of the plant's life span, it is expected that the amount of assimilation material to be transported to the grains by photosynthesis will increase and the grain weight in the spike will increase. However, it is thought that due to the change of climatic factors that change from year to year, it causes a decrease in ear grain weightsThe highest grain weight per spike was determined as 1.099 (g) from 300 seeds m^{-2}. This was followed by 1.059 (g) from 500 seeds m^{-2}. The lowest grain yield per spike was found 0.972 (g) from 600 seeds m^{-2} seeding rates.

Table 8. Grain weights per spike at different row spacings and seeding rates

	Seeding Rates (seed m^{-2})				
Row Spacing	300	400	500	600	Avareage
15 cm	1.193	1.073	1.053	1.090	1.103
17.5 cm	1.187	1.063	1.160	0.903	1.078
20 cm	1.097	1.077	1.037	0.930	1.035
22.5 cm	1.077	1.027	1.080	1.020	1.051
25 cm	0.943	1.027	0.963	0.917	0.963
Avareage	1.099 a*	1.053 ab	1.059 a	0.972 b	

*The difference between the means with the same letter is insignificant (Duncan, $p \leq 0.05$)

It is seen that as the seeding rates increases, the grain yield per spike decreases. Mcleod, (1982); Kazan and Doğan, (2005); Öztürkci, (2009); Yağmur et al., (2021) determined that the effect of increasing sowing rates on the grain weight in the spike was affected negatively. In case of seeding rates, plants compete with each other due to the use of light, water and nutrients. Due to the fact that the spike are weak and small as a result of competition, it is thought that the grains formed in the spike are also small grained, which will

reduce the grain weight in the spike (Kazan and Doğan, 2005). In addition, the lack of precipitation in the period after the spike causes the plants to be affected by water stress, which negatively affects the grain weight and grain yield (Yanbeyi and Sezer, 2006).

Grain Yield

The results of the analysis of variance related to grain yield is examined, the effect of row spacing and row spacing X seeding rates interactions on grain yield is significant at $p<0.05$ level, while the effect of seeding rates on grain yield is significant at $p<0.01$ level. The highest grain yield was obtained as 349.6 da/kg from 15 cm row spacing, the lowest grain yield average was determined at 270.0 da/kg from 25 cm row spacing.

It can be seen from the results of the study that the grain yield decreased as the row spacing was increased. According to the results of the research, it was determined that the average grain yield of the row spacing was statistically significant and the grain yield decreased as the spacing distance increased. While the highest grain yield was obtained at 15 cm row spacing (349.61 kg da^{-1}), the lowest grain yield was obtained from the widest row spacing (270.05 kg da^{-1}) with 25 cm. Contrary to this study, Iqbal et al. (2010) determined that the interaction of row spacing with grain yield was insignificant and the highest value of grain yield was at 22.5 cm row spacing. Önmez, (1997) investigated the effects of row spacing on yield and yield components in two rye cultivars in Konya Karapınar arid conditions and reported that the highest grain yield was reached in 16 cm row spacing in both cultivars. Moreover, Ulukan and Kün, (2007) in which they investigated the effects of row spacing (5 cm, 10 cm, 17.5 cm) on the yield of wheat varieties in Ankara conditions, they found that the grain yield per square meter was higher in narrow row spacing.

Table 9. Grain yield at different row spacings and seeding rates

	Seeding Rates (seed m^{-2})				
Row Spacing	300	400	500	600	Avareage
15 cm	248.3 ghı*	352.0 bcd	387.6 ab	410.4 a	349.6 a
17.5 cm	262.7 fghı	360.4 abc	327.7 cde	350.2 bcd	325.2 ab
20 cm	245.9 ghı	302.7 cdefg	333.7 bcde	314.7 cdef	299.2 bc
22.5 cm	242.9 hı	300.6 cdefgh	295.7 defghı	309.8 cdef	287.2 bc
25 cm	237.5 ı	289.1 efghı	304.1 cdefg	249.4 ghı	270.0 c
Avareage	247.4 b	320.9 a	329.7 a	326.9 a	

*The difference between the means with the same letter is insignificant (Duncan, $p \leq 0.05$)

Gençtan and Balkan, (2008) investigated the effects of two different sowing densities and four different row spacing (17, 34, 51 and 68 cm) on the yields of three bread wheat varieties, and found that the highest grain yield was 17 cm row spacing. They reported that the grain yield decreased with the increase of the row spacing. Öztürkci, (2009) found that as the row spacing increased, the grain yield decreased, and the highest grain yield was obtained from 15 cm row spacing (246.55 kg da^{-1}), while the lowest grain yield (183.11 kg da^{-1}) was obtained from the widest row spacing of 30 cm. These findings support our findings that the narrowing in row spacing causes an increase in grain yield.

Considering Table 9, where the averages of seed yields are given; The highest grain yield was determined as 329.7 da/kg from 500 seeds m^{-2} seeding rate, followed by 326.9 da/kg with 600 seeds m^{-2} . The lowest grain yield was found as 247.4 da/kg with 300 seeds m^{-2} seeding rates. Lloveras et al,. (2004); Teich and Smith, (1993) reported that as increasing of the seeding rate increased the grain yield per unit area.

The effect of row spacing X seeding rate interaction on grain yield was statistically significant. It was determined that the highest grain yield was 410.4 kg da^{-1} with 600 seeds m^{-2} seeding rates and in 15 cm row spacing. The lowest

grain yield was determined as 237.5 kg da^{-1} with 300 seeds m^{-2} seeding rate in 25 cm row spacing.

CONCLUSION

It concluded that the grain yield increased as the seeding rate was increased. the highest grain yield was obtained from 500 seeds m^{-2} seeding rates, while the lowest value was obtained from 300 seeds m^{-2} seeding rate, which is the lowest seeding rate. Moreover the grain yield decreased as the row spacing was increased. As a result, the highest grain yield was obtained 349.61 kg da^{-1} at 15 cm row spacing, the lowest grain yield was obtained from the widest row spacing (270.05 kg da^{-1}) with 25 cm.

Research evaluations are based on one-year data. It is thought that similar studies to be carried out in Kırşehir and the ecology of the region will increase the reliability of the interpretations by providing more information on the subject.

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CHAPTER 3

BIO-STIMULATORS, AN EXCELLENT OUT-POINT FOR SUSTAINABLE HORTICULTURE?

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1. INTRODUCTION

The two most important requirements in horticulture are sustainability and meeting the consumer's demand for safe food. These two factors have been gaining more and more importance in recent years in the production processes from the garden to the table.

Especially, the increasing polluting effects of agricultural production on the environment necessitate both cheaper and environmentally friendly alternative solutions for growers. Many purposes and reasons such as increasingly polluted underground and surface waters, decreasing clean water resources, deterioration in soil structure caused by wrong and excessive applications, reducing all chemical inputs by preserving crop quality and yield, and restoring the physical, chemical and microbiological balance of the soil. encourages the search for alternative solutions (Basile et al. 2020). Moreover; Increasing and maintaining productivity in species that are approaching the end of their genetic capacity and arable lands that are gradually decreasing are also prominent issues (Muhie 2022). Among these searches, the results that the common denominator is biostimulant gain importance.

Many biological stimulating products used for different purposes in different parts of the world in the early stages of agriculture, when the chemicals required were not included in the production processes, a clearer understanding of the problems caused by chemical inputs, their effects on human health and the clarification of the mechanism of action of these products in the light of technological developments. It also created the conditions that make these applications more attractive.

The term bio-stimulant; It has also been expressed as a group of compounds that can be effective even at very small doses, except for fertilizer or any pesticide group (Du Jardin 2012). Bio-stimulators of the European Biostimulant Industry Council (EBIC); defined as substances that, when applied to plants or the rhizosphere, whose function is to promote natural processes that independently support nutrient uptake, productivity, tolerance to abiotic stress and/or crop quality (Anon 2022a). The organization called the biostimulant Coalition in the USA includes these items; defined as primary or secondary substances or compounds other than micronutrients that can be shown by scientific research to be beneficial to plant species when applied externally (Anon 2022b). However, there is still no globally accepted definition

of biostimulators. Therefore, substances known and accepted as bio-promoters are used in combination with other nutritional elements. In studies related subject, humic substances, complex organic substances, useful chemical elements, inorganic salts including phosphite, seaweed extracts and some plant extracts, chitin and chitosan derivatives, antiperspirants and free amino acids and N-containing substances that act as biostimulant and beneficial microorganisms (such as arbuscular mycorrhizal fungi and plant growth-promoting rhizobacteria) are considered biostimulant (du Jardin 2015). Such a large number of substances of biological origin have been subjected to various definitions by different researchers. Indeed, Abbott et al (2018), biostimulators; classified as bio promoters, organic substances and microbial inoculants.

In addition, it has been observed that biostimulators can be single or multi-component, but there are also synergistic effects of several different components. (Kisvarga et al 2022).

The purpose of this revew is to contribute to a better understanding of plant biostimulators on the basis of knowledge about the classification of bio-stimulating products used in agriculture and horticulture.

2. BIO-STIMULATING SUBSTANCES

Lots of studies have been carried out to identify products that can be used in aquaculture to improve yield and quality in horticultural crops, and recently to help overcome different types of environmental stresses. Global demands for environmentally friendly product management practices, safe food sustainability and the need for high yields are encouraging factors in these studies. The very limited use of chemical fertilizers and pesticides in organic agriculture and good agricultural practices has increased the interest both in the improvement of plants that can adapt to these new conditions more easily and in the possibilities of using bio-stimulators in overcoming the risks posed by climatic variations. As a matter of fact, it has been reported that these products, which are briefly defined as "formulated products of biological origin" (Yakhin et al 2017), can be applied to soil or leaves (Kunicki et al 2010). Although they have common features such as being of different nature, being organic or inorganic molecules, and acting with very different physiological functions (increasing photosynthetic activity, antioxidant activity, increased tolerance to abiotic stresses, promoting lateral roots, increasing nutrient utilization

efficiency) expanding studies on the subject also reinforces the idea that modes of action will gain importance in the identification and detection of biostimulators (du Jardin 2015, Bulgari et al 2019).

Below, some of these substances, which have been defined as bio-stimulating in scientific studies carried out so far, have been classified and evaluated in terms of their mechanism of action and usage areas.

2.1. Humic and Fulvic Acids

Humic substances (HS) consist of chemical and biologically transformed products of plant and animal wastes and microbial metabolisms. Thanks to their content, they contribute to the regulation of many important ecological and environmental processes. In particular, it regulates plant growth and maintenance of terrestrial life, soil carbon and nitrogen cycle, especially microorganism diversity and increase, and stabilization of soil structure (Piccolo, 2012). It has been demonstrated that humus material is essentially self-assembled supramolecular assemblies of small heterogeneous molecules held together by weak hydrophobic linkages (Piccolo, 2002) suggesting that humic substances in solution form diverse, relatively low molecular mass, dynamic relationships that are stabilized by hydrophobic interactions and H-bonds defined as a collection of components. Soil scientists define humic acids as humus materials that dissolve in aqueous alkaline solutions but precipitate when pH is adjusted to 1-2, while fulvic acids remain in solution after alkaline aqueous extracts are acidified. Chemically, humic substances are a product of a saponification reaction with alkaline extraction from soils and sediments (Canellas et al 2015).

It is known that the plant's responses to HS also differ depending on the plant species and development period, application method and speed, source of HS and environmental conditions (Trevisan et al. 2010a,b). Studies have shown that plant physiological responses to HS isolated from brown coal are less than those observed in response to the addition of HS isolated from peat, compost or vermicompost (Canellas and Olivares 2014). Humic substances make up more than 60% of soil organic matter and are the main component of organic fertilizers and are known to contain significant amounts of nutrients. Humic substances act as biostimulant through their interaction with plant membrane transporters responsible for nutrient uptake and membrane-

associated signal transduction cascades that regulate growth and development.

Moreover, it is reported that most of the reported beneficial effects of humic substances on plant growth are related to their positive effects on changes in root architecture (Rouphael et al 2015) (Fig 1).

Application to plants with humic matter allows the fine roots to directly change the pH of the soil surrounding the root (rhizosphere) through the release of H^+ ions (via H^+-ATPase) and organic acids (such as citrate, oxalate). Proton pumping across the root plasma membrane generates the proton motive force necessary to promote active and passive transport of ions and metabolites along the symplastic pathway (Morsomme & Boutry, 2000) (Fig 2).

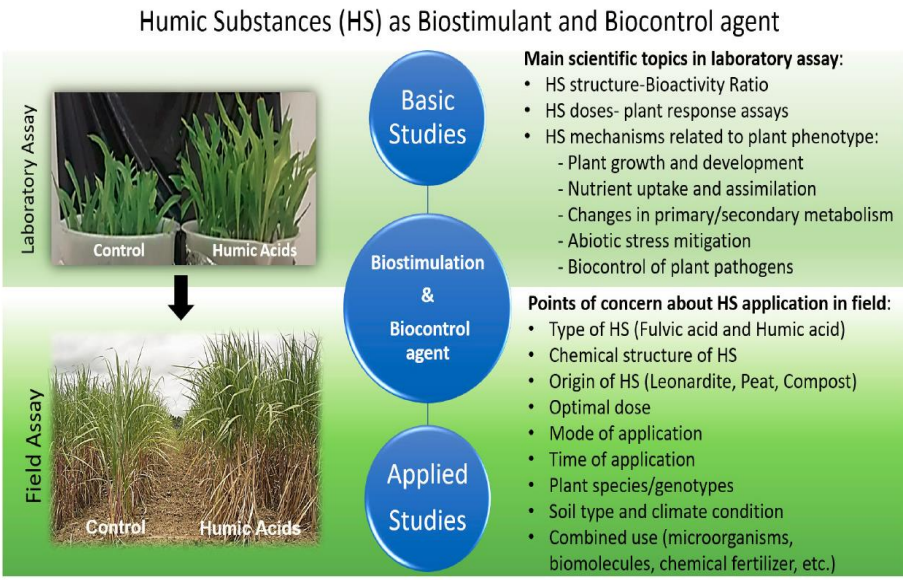

Figure 1. Advantages and limitations of humic substance application under open-fields and greenhouse conditions (Jindo et al. 2020).

According to the acid growth theory proposed to explain the amplifying effect of humic substances, small bioactive exogenous organic molecules (such as IAA) access cell receptors to trigger cell signaling. The decreasing pH of the cell wall activates enzymes that relax the cell wall and initiates cell volume expansion (Hager 2003). As a matter of fact, some of the auxin-like molecules are present in humic substances. This is the most important evidence explaining the mechanism of root growth induction of humic substance applications (Zandonadi et al, 2007). The increase in organic acid exudation in many

products following the application of humic substances contributes to this result (Puglisi et al. 2008). Humic substances induce H^+ -ATPase activity, which in turn energizes secondary ion transporters and promotes nutrient uptake.

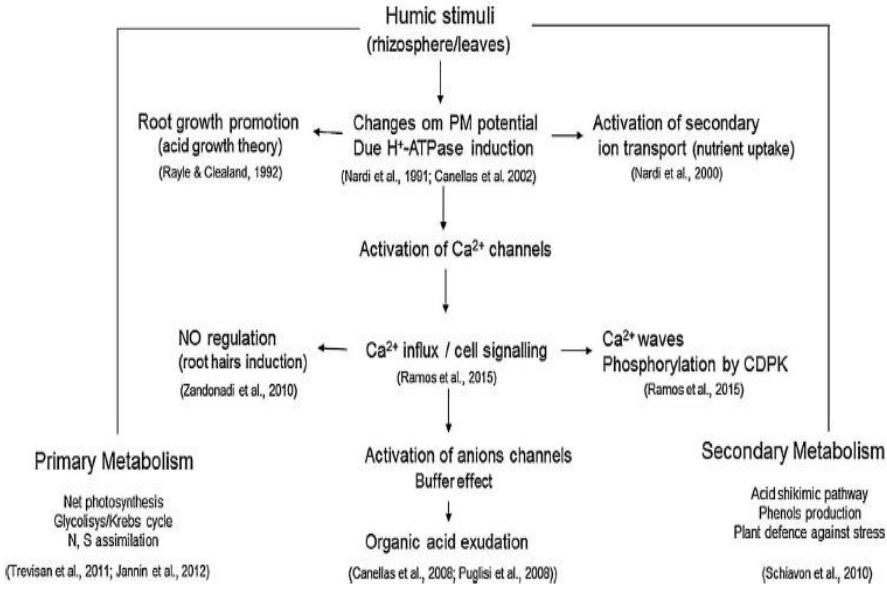

Figure 2. General picture showing the modes of action of humic substances, in line with the acid growth theory, as elicitor of cell signalling by induction of plasma membrane (PM) H+-ATPase. NO = nitric oxide; CDPK = calcium-dependent protein kinase (Canellas et al. 2015).

Transport of nitrate across the plasma membrane is facilitated by ion channels. That is, HS can promote plant growth through induction of carbon and nitrogen metabolism (Rouphael et al 2015). It has also been reported that humic substances negatively affect the activity of enzymes such as glucokinase, phosphoglucose isomerase, aldolase and pyruvate kinase in glucose metabolism (Nardi et al 2009). From the sequence analysis and gene ontology classification, it was concluded that many genes involved in developmental and metabolic processes as well as transcription regulation or RNA metabolism are regulated by humic substances. Furthermore, 80% of the genes involved in sulfate metabolism were determined to be up-regulated by humic substances, with a high representation of genes involved in sulfate uptake and assimilation (sulphate transporter, ATP sulfilase and serine acetyltransferase).

Humic matter effects confirmed in this way will ultimately lead to an increase in net photosynthesis rate and production (Trevisan et al, 2011). Besides significant changes in plant primary metabolism and nutrient intake, HS can also strongly affect secondary metabolism. (Schiavon et al 2010). Humic substances have been proven to increase the expression of phenylalanine (tyrosine) ammonialyase (PAL/TAL), which catalyzes the first major step in the biosynthesis of phenolics by converting phenylalanine to trans-cinnamic acid and tyrosine to p-coumaric acid. PAL/TAL expression is accompanied by phenol accumulation in leaves.

Studies have shown that the stimulating effects of stimulant substances on plant secondary metabolism provide an innovative approach to explore plant responses to stress (Oliveras et al 2015, Hernandez et al 2015).

One of the most important uses of humic substances in horticultural cultivation is their positive effects on alleviating drought stress. In plants exposed to drought stress to which humic substances are applied, osmotic adjustment capacity is observed by maintaining water absorption and cell turgor (Azevedo and Lea, 2011). Enzymatic and non-enzymatic antioxidant defense mechanisms are used by plants to scavenge/detoxify ROS. Peroxidase enzyme is a common scavenger plant enzyme involved in the regulation of oxidative stress. Indeed, studies have shown that humic substances increase peroxidase activity (Pizzaghello et al. 2001, Kellos et al. 2008, Márquez-García et al. 2011, García et al. 2014).

Excessive amounts of heavy metals in soils are a source of abiotic stress for plants. Uptake of heavy metals by plants is critically dependent on the mobility and availability of metals in the soil, which is affected by the amount of solid and dissolved organic matter present, respectively. The carboxylic and phenolic hydroxyl groups of humic substances are the main binding sites for metals (Zeng et al. 2002). Many studies have shown that fulvic acids can alleviate Pb phytotoxicity by complexing highly toxic free Pb^{2+} in solution and thus reducing Pb uptake (Haghighi et al. 2010, Shahid et al. 2012, Ouni et al. 2014).

Soil salinity is one of the most important problems in horticultural cultivation, especially for some species, reducing yield and quality in arid and semi-arid regions of the world. The basis of the negative effects includes the deterioration of plant water retention due to the high osmotic potential of the

external environment and its negative effects on gas exchange, photosynthesis and protein synthesis (Romero-Aranda et al. 2001).

Humic substances can be effective indirectly by contributing positively to the physical, chemical and microbiological properties of soils. Its direct effects on the plant are due to its positive contributions to germination, plant growth (root and shoot) and hormone-like activity. Humic substances can induce salt tolerance through root growth, differentiation of mineral uptake and reduction of membrane damage (Türkmen et al. 2004, Paksoy et al. 2010, Canellas et al. 2015).

The effectiveness of humic substances in horticultural cultivation can vary depending on many factors such as concentration, plant type, application method. Growth and development of humic substances (Boyhan et al. 2001, Selim and Mosa, 2012, Naidu et al. 2013, Denre et al. 2014, Leventoğlu and Erdal 2014, Cansu and Erdal 2018, Jindo et al 2020, Turan et al. 2022) , diseases (Zaller 2006, Singh et al. 2010, Olivares et al. 2015, Jindo et al. 2020), effects on marketable product (Boyhan et al. 2001, Neri et al. 2002; Naidu et al. 2013, Farahi et al. . 2013, Denre et al. 2014, Turan et al. 2022) activities have been carried out. Studies have shown a better crop response using humic-like substances from compost or vermicompost rather than humic extracts from leonardite (Azcona et al. 2011).

Recently, HS have begun to be sold as plant growth accelerators or as biological control agents in mixtures with beneficial microorganisms (Olivares et al. 2015). This new method of application is that HS are used as carriers to more easily introduce beneficial microorganisms into the plant, thanks to their resistance to microbial activity. This new concept of biofertilizer shows significant positive effects on crop yield (Canellas et al. 2015). It has been reported in many studies that concomitant use promotes the increase of water-soluble P and total nitrogen content (Busato et al. 2012, Olivares et al. 2015). Thus, application of humates isolated from vermicompost in combination with diazotrophic endophytic bacterial inoculation seems to be a powerful biotechnological tool to promote plant growth in sustainable farming systems. Applications of humic substances in horticultural cultivation; yield increases, increases in lateral rooting, increase in nutrient uptake efficiency, positive effects on climate adaptation, marketable fruit increase, early flowering, improvement in tuber development, increase in fruit aroma substances, fresh

and dry shoot-root weight increases and positive effects on seed germination parameters. Different results have been reported in various horticultural species (Neri et al. 2002, Farah et al. 2013, Kandil et al. 2013, Denre et al. 2014, Olivares et al. 2015, Cansu and Erdal 2018, Jindo et al. 2020, Turan et al. 2022).

2.2. Seaweed Extracts

Seaweeds are green, brown and red marine microalgae that help nourish marine ecosystems by improving the properties of seawater (Bhattacharyya and Jha 2012). A relatively small proportion of total seaweed species are of significant importance in agriculture as animal and human food/supplements, as well as as mulch/fertilizer and modified extracts (Craigie 2011).

It has been reported that seaweed extracts can be used in various ways such as seed treatment, foliar and soil application for the promotion of plant growth. They are more valuable products, especially since they do not contain toxic substances and are biologically and environmentally friendly. Therefore, the use of seaweed extracts in sustainable fruit growing is increasing (Rana et al. 2022). Its application promotes improvement of plant growth, nutrient incorporation, fruit set, resistance to pests and diseases, and stress management such as drought, salinity and temperature (Yakhin et al. 2017, Rouphael and Colla 2020). The most common brown seaweed species on the market are *Ascophyllum* (*Ascophyllum nodosum*), sea bamboo (*Ecklonia maxima*), giant algae (*Macrocystis pyrifera*) and southern bull algae (Durvillea potato). Species known as brown seaweeds also contain active secondary metabolites and vitamin precursors. In addition, high antioxidant activity may be the common feature of extracts of these species.

A number of betaine and betaine analogs have been identified in seaweed extracts (MacKinnon et al. 2010). Seaweed extracts are obtained from these species by different processes. Therefore, the nutrient composition in seaweed extract varies depending on the seaweed species and extraction method (such as alkaline extraction, acid extraction, and cell bust technology) (Khairy and El-Shafay 2013). Therefore, seaweed extracts are now much more widely accepted as "plant biostimulators". In general, seaweed extracts, even at low concentrations, can induce a series of phytophysiological responses, such as promoting plant growth, improving flowering and yield, as well as improving the quality of products, improving the nutrient content and shelf life of the

edible product. It has also been reported that applications of different types of extracts increase the tolerance of plants to a wide variety of abiotic stresses, namely salinity, drought and temperature extremes (Fig. 3). In addition to betaines, brown seaweed extracts are reported to contain amino acids. One of the main components of commercial extracts of all seaweeds is polysaccharides. These may constitute 30-40% of the extract on a dry weight basis (Rayorath et al. 2009). Common polysaccharides found in brown seaweed extracts include alginates, fucoidans, laminarans, lichen-like glucans, and fucose-containing glucans (Khan et al. 2009). Also known as alginates, they are polymers of mannuronic and guluronic acids whose viscosity varies depending on the type of seaweed. Laminarins, on the other hand, are precursors that provide plant defense responses against fungal and bacterial pathogens (Mercier et al. 2001). Especially brown seaweeds are rich in phenolic compounds. Phenolics are secondary metabolites synthesized under stress that protect cells and cellular components (Wang et al. 2009). Especially brown seaweeds such as *Fucus vesiculosus*, *F. serratus* and *Ascophyllum nodosum* have high total phenolic concentrations (Keyrouzet et al. 2011, Balboa et al. 2013). In addition, seaweed extracts contain numerous phytohormones, including auxins, cytokinins, gibberellins, abscisic acid and brassinosteroids (Stirk et al. 2014).

Brown seaweeds such as *Alaria esculenta*, *Ascophyllum nodosum*, *Ectocarpus siliculosus*, *Fucus serratus*, *F. spiralis*, *F. vesiculosus*, *Halidrys siliquosa*, *Laminaria saccharina* and *Pilayella littoralis* L. contain mannitol as an important protective compound in response to abiotic stress factors. Mannitolis is also known as a chelating agent, explaining why seaweed can release unavailable elements from the soil (Reed et al. 1985). Seaweed extracts can be in liquid or soluble solid powder form. It is used with drip irrigation and in the form of foliar spray. Especially in soil applications, the results such as improving the water holding capacity of the soil (Lattner et al. 2003) and the improvement of root growth and soil microbiology are quite evident (Chen et al. 2003). It has been reported that thanks to the vitamin K1 it contains, it can also provide acidification of the rhizosphere by changing the plasma membrane proton pumps (Luthje and Bottger 1995). It is stated that it promotes the expression of some genes (such as NRT1.1) that improve nutrient intake.

In this way, results such as root growth, increased root efficiency and improved nitrogen assimilation occur (Castaings et al. 2011, Battacharyya et al. 2015).

Many seaweed extracts are known to promote the growth of beneficial fungi in the soil and root colonization. As a matter of fact, alginic acid, the most important compound in these extracts, promotes the growth of hyphae of AMFs. As a result, the improvement of phosphorus nutrition in plants occurs (Kuwada et al. 2006, Battacharyya et al. 2015). It has been reported that the chemical components of *A. nodosum* extract induce amylase activity in seeds, which is independent of gibberellins (GA_3), and that seaweed extracts may also contain compounds that are structurally different from phytohormones, however, they induce physiological responses resembling phytohormones (Rayorath et al. 2008). Flavonoids, which are abundantly present in brown seaweed, play an important role in plant development against UV rays and other abiotic and biotic stress conditions. Chalcone isomerase, which is increased by the application of seaweed extracts, is a key enzyme in the biosynthesis of phenyl propanoid plant defense compounds, which are flavanone precursors. As a matter of fact, the applications also provided an increase in chlorophyll in plants. This increase; The increase in biogenesis of chloroplasts is due to the decrease in chlorophyll degradation and delay in aging (Spinelli et al. 2010, Nair et al. 2012, Jannin et al. 2013).

2.3. Chitosan

Chitosan, which is an important tool for inhibiting microbial growth and reducing microbial membrane integrity, is the diacetylated form of chitin biopolymer, which has been extensively studied. Chitin and chitosan are copolymers of N-acetyl-d-glucosamine. Although it exists in many sources in nature, it is produced from shrimp and crab shells by demineralization and deproteinization (Younes and Rinauda 2015).

Chitosan applications in plants provide an oxidative flash effect with hydrogen peroxide production in plant tissues (Zhao et al. 2007). This leads to the promotion of plant defense enzymes such as phenylalanine ammonia-lyase (PAL) (Pichyangkura and Chadchawan 2015). This effect has been determined in many horticultural crops (Meng and Tian 2009, Badawya and Rabea 2009, Ghasemnezhad et al. 2010).

In particular, hydrogen peroxidase production is known to trigger the ROS scavenging system and the expression of other oxidative stress sensitive genes. Several enzymes in the ROS scavenging system upregulate antioxidant systems such as superoxide dismutase (SOD), peroxidase (POD), and CATb (Desikan et al. 2001). It has been reported that chitosan application can reduce stomatal opening through hydrogen peroxide signaling in some horticultural crops such as tomatoes (Lee et al. 1999).

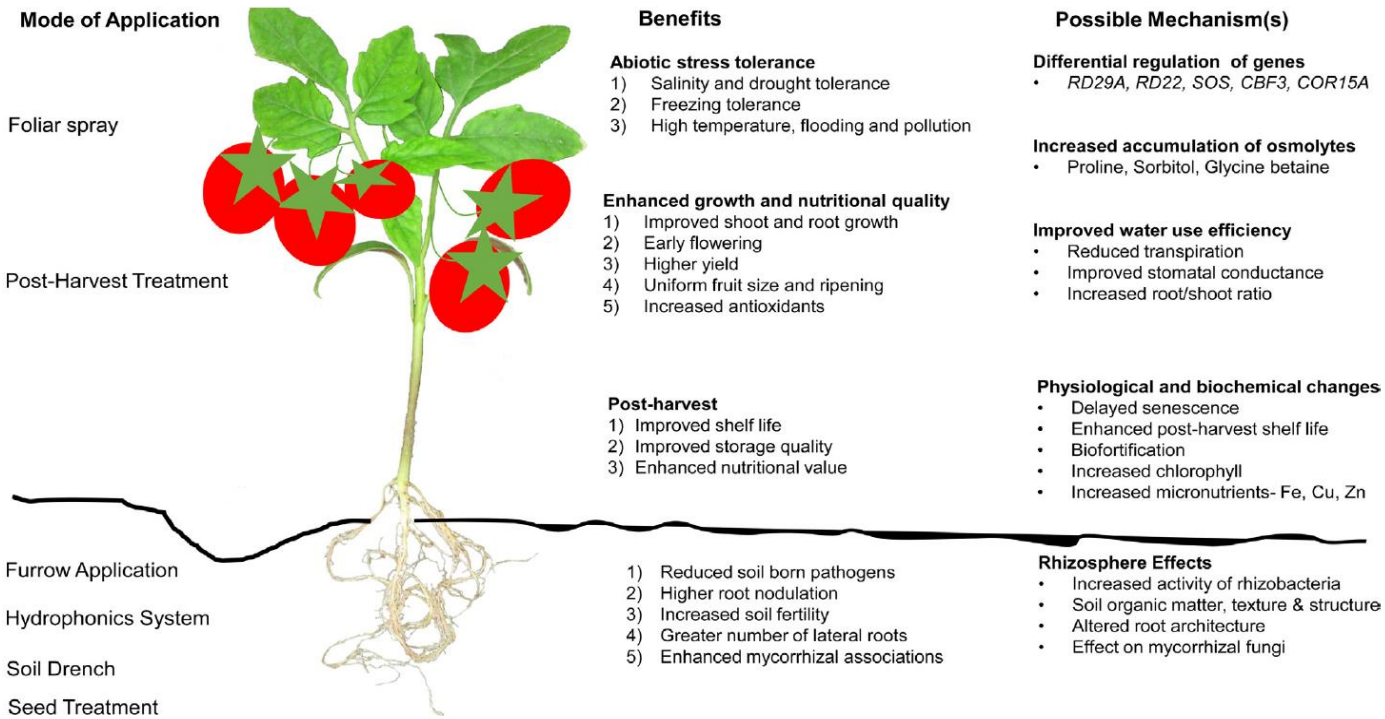

Figure 3. Schematic diagram depicting methods of application of seaweed extracts, and their effects on plant and mechanisms of action (Battacharyya et al. 2015).

It has been reported that the application of chitosan together with hydrogen peroxide scavengers such as CAT and ascorbic acid also causes inhibition of stomatal closure (Younes and Rinauda 2015).

In foliar applications (*Capsicum* sp.), biomass and yield were preserved, although the plant reduced water use and transpiration rate (Bitelli et al 2001). Significant increases were observed in ABA and hydrogen peroxide levels in beans (Iriti et al 2009).

Chitosan induces chitinase and glucanase in many horticultural crops such as grape (Trotel-Aziz et al. 2006), pear (Meng et al. 2010), peach (Ma et al. 2013), tomato (Sathiyabama et al. 2014). The increase in glucanase or chitinase levels in plants parallels increased disease resistance, suggesting the idea that chitosan-induced disease resistance is potentially based on induction of these enzymes.

Chitosan applications on okra and grapevines also increased the chlorophyll content (Górnik et al. 2008, Mondal et al. 2012). H_2O_2 production after chitosan application is important for inducing drought tolerance. Similarly, the application of chitosan with appropriate concentration to the seed induced salt tolerance (Pongprayoon et al. 2013, Mahdavi 2013). These results show that hydrogen peroxide acts as a signal molecule in the activity of chitosan in many plant species, the octadecanoid pathway is followed in the production of H_2O_2, and the enzymes working in this pathway are localized in the chloroplasts (Schaller and Stintzi 2009). The thesis that chitosan differs in foliar applications by causing changes in the expression of many genes in the most important organelle, the chloroplast, has gained weight with the results of the study (Limpanavech et al. 2008).

Briefly, the cellular responses to chitosan applications can be summarized as follows: chitosan binds to the plant cell membrane and initiates a secondary messenger signal to the cell, producing hydrogen peroxide (H_2O_2) via the octadecanoid pathway and nitric oxide (NO) in the chloroplast. While H_2O_2 triggers both reactive oxygen species scavenging system and abscisic acid (ABA) synthesis pathway, NO regulates phosphatidic acid (PA) synthesis via phospholipase C (PLC) and diacylglycerol kinase (DGK) (PLC/DGK) pathways. PA increases the effect of ABA by inhibiting ABI1 (negative regulator of ABA).

Then, ABA induces stomatal closure and other abiotic stress responses. H_2O_2 coordinates the activity with jasminic acid (JA) synthesized via the octadecanoid pathway and regulates the expression of biotic stress sensitive genes (chitinase or glucanase (Pichyangkuraa and Chadchawan 2015) (Fig. 4).

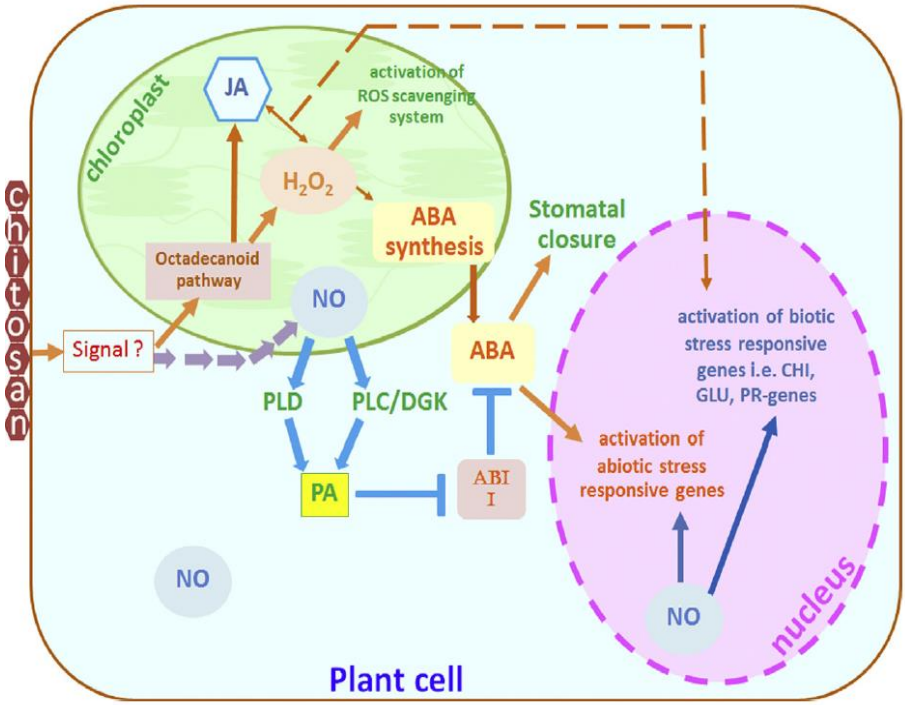

Figure 4. Model of chitosan responses at the cellular level (Pichyangkuraa and Chadchawan 2015).

As with plant hormones, chitosan concentration affects each plant response specifically (Lin et al. 2005, Pornpienpakdee et al. 2010, Mathew and Sankar, 2012). Also, the same plant species at different developmental stages may respond differently to each chitosan species (Pornpienpakdee et al. 2010).

2.4. Protein Hydrolysates and other N-containing compounds

Protein hydrolysates (PH) are a group of plant biostimulators produced by enzymatic and/or chemical hydrolysis and containing a mixture of peptides and amino acids. They may also contain other compounds that may contribute to their biostimulant effects, such as carbohydrates, phenols, mineral elements,

phytohormones, and other organic compounds. In short, PHs are mixtures of polypeptides, oligopeptides and amino acids produced from protein sources using partial hydrolysis (Schaafsma 2009).

Protein hydrolysates have shown a wide range of benefits in many crop plants and have the ability to affect multiple plant functions. Animal residues known as phs include skin by-products, blood meal, fish by-products, animal epithelial or connective tissues such as chicken feathers, and casein. Herbally, it includes leguminous seeds, alfalfa grass, corn wet milling and herbal by-products (Colla et al. 2015).

PHs play key roles as biostimulants through the modulation of plant molecular and physiological processes that trigger growth, increase yields and alleviate the impact of abiotic stress on crops (Calvo et al. 2014, Yakhin et al. 2017). These activities include salinity, heavy metal, high temperature stress, food stress and water stress (Cerdán et al. 2013, Colla et al. 2013). The direct effects of pHs on plants include the stimulation of carbon and nitrogen metabolism, as well as the regulation of N uptake mediated by key enzymes involved in the N assimilation process and the regulation of the activity of citrate synthase, isocitrate dehydrogenase and malate dehydrogenase enzymes involved in the tricarboxylic acid cycle (du Jardin 2015, Nardi et al. 2016).

These substances can also interfere with hormonal activities thanks to the bioactive peptides in their content (Colla et al. 2014, 2015).

Many studies in cultivated plants have proven that many commercial products from PHs elicit hormone-like activities (auxin and gibberellins), promoting root and shoot growth and thus crop productivity (Colla et al. 2014, Lucini et al. 2015, Carillo et al. 2019, Choi et al. 2022).

Leaf and root applications increase the uptake and utilization efficiency of both macro and micronutrients (Colla et al. 2015, Carillo et al. 2019), and this activity is generally associated with modifications in root architecture (density, length and number of lateral roots), as well as nutrient availability. It has been reported to be Associated with increased nutrient availability in the soil solution resulting from its complexation by peptides (du Jardin 2015, Caruso et al. 2019).

The biostimulating effect of PHs may vary depending on their origin and characteristics, species, cultivar, phenological stages of plants, growing conditions, concentration, application time and mode (leaf-root), solubility and

leaf permeability. The penetration of active ingredients (amino acids and peptides) into the internal structures of plants is very important when the PH-based biostimulant is usually applied foliar (Colla et al. 2015, Yakhin et al. 2017).

It is accepted that PHs can improve plant growth by changing the physiological and developmental processes of microorganisms, increasing nutrient and water intake as well as increasing resistance to environmental stress factors (Philippot et al. 2013).

Organic molecules at PHs can be considered a source of carbon and nitrogen for microorganisms living in the rhizosphere and phyllosphere of plants. Also, microorganisms are generally more competitive than plants for amino acids (Moe 2013).

It was concluded that when soybean meal, which is one of the pH's, is used as seed coating material, it enhances some key enzymes involved in nitrogen metabolism, triggering nitrogen uptake, assimilation and translocation, thereby improving various growth characteristics. It was concluded that the presence of tryptophan, which is the main precursor of IAA biosynthesis and bioactive peptides, a significant auxin-like activity occurs (Colla et al. 2014).

The hormone-like activities of PHs have been attributed to the fact that PHs contain a variety of small peptides such as signaling molecules that can modulate a pathway involved in the synthesis of an endogenous phytohormone (Matsumiya and Kubo 2011). In many studies with PHs, fruit number increases in horticultural crops have been reported.

It has been stated that the increase in fruit number may be due to multiple effects. Hormone-like activity at pH and better pollen viability from amino acids is one of them (Francesca et al. 2020). Also, indirect effects of PHs may be due to improved photosynthetic capacity and thus better source-to-sink ratio with more photosynthesizing to fruit clusters. As a matter of fact, in other studies, chlorophyll content and photosynthesis increased in plants treated with PHs (Choi et al. 2022).

Protein hydrolysates have great potential to improve crop performance, especially under environmental stress conditions. Root applications of PHs have been shown to be beneficial by increasing nutrient utilization efficiency, increasing nutrient availability, root growth, nutrient uptake and assimilation in

a variety of crops. In addition, foliar and root (substrate wetting) applications of PHs improve seed germination, plant growth, fruit set and growth. It exhibits hormone-like activities (particularly auxin-like and gibberellin-like activity) that cause cleavage.

In addition, the indirect effects of PHs may be due to improved photosynthetic capacity and thus better source-to-sink ratio with more photoassimilation to fruit clusters. As a matter of fact, in other studies, chlorophyll content and photosynthesis increased in plants treated with PHs (Choi et al. 2022).

Protein hydrolysates have great potential to improve crop performance, especially under environmental stress conditions. Root applications of PHs have been shown to be beneficial by increasing nutrient utilization efficiency, increasing nutrient availability, root growth, nutrient uptake and assimilation in a variety of crops. Furthermore, foliar and root (substrate wetting) applications of PHs exhibit hormone-like activities (particularly auxin-like and gibberellin-like activity) leading to stimulation of seed germination, plant growth, fruit set and growth. PHs not only increase yield but also improve some quality parameters such as fruit size, skin colour, soluble solids and antioxidant contents. At the same time, PHs have great potential to reduce nitrate accumulation in leafy vegetables such as lettuce, spinach and arugula. However, the mechanisms regulating the beneficial effects of PHs on plants are not fully understood.

It is reported that combining PHs with certain microbial taxa, which are well known for their potential to help plants obtain nutrients and withstand biotic and abiotic stress, will further benefit plants (Choi et al. 2022).

2.5. Inorganic Compounds

While not essential to all plants, chemical elements that can promote plant growth are beneficial elements. Useful elements include selenium, silicon, cobalt, aluminum and sodium. These elements are found in soil and plants as inorganic salts and even insoluble salts such as amorphous silica.

The five main beneficial elements are Al, Co, Na, Se and Si and are found in soil and plants as different inorganic salts and in insoluble forms such as amorphous silica ($SiO_2.nH_2O$) in gramineal species. The benefits of these elements are not limited to their chemical structures, but are considered together

with their positive effects on plant growth and stress response (Azad et al. 2021, Franzoni et al. 2022). Besides these substances, phosphites can also be classified in this group.

Phosphorus plays an important role in genetic inheritance (nucleic acids DNA and RNA), structural membrane, signal transduction pathways, and cell metabolism to all life forms on earth, including both lower and higher plants (Gómez-Merino and Trejo-Tellez 2015) . Phosphite (Phi) is a reduced form of phosphate that is considered a unique biostimulator in horticulture, especially in fruit culture. It has been investigated in many studies that the phosphite and conjugated form of phosphorus is good for plant nutrition and is used as pesticide, additional fertilizer, and biostimulator (López-Arredondo et al. 2014).

Phosphites can improve nutrient absorption and uptake, tolerance to abiotic and biotic stress, and product quality (Burra et al. 2014, Rana et al. 2022).

The effects of inorganic biostimulant substances include cell wall solidification, osmoregulation, reduced transpiration by crystal deposits, thermal regulation by radiation reflection, enzyme activity by co-factors, plant nutrition through interactions with other elements during uptake and motility, antioxidant protection, interactions with symbiotic microorganisms, pathogen and herbivorous response, protection against heavy metal toxicity, plant hormone synthesis and signaling (Pilon-Smits et al. 2009). Inorganic salts of these substances (chlorides, phosphates, phosphites, silicates and carbonates) have been used as fungicides (Deliopoulos et al. 2010). Although their mode of action has not yet been fully determined, these inorganic compounds affect osmotic, pH and redox homeostasis, hormone signaling, and enzymes involved in the stress response. Its functions as a biostimulant of plant growth affect nutritional efficiency and abiotic stress tolerance, thus, unlike its fungicidal effects and its functions as fertilisers as nutrient sources, it deserves more attention (du Jardin 2015)

2.6. Benefical Bacteria

Bacteria are small, microscopic, single-celled organisms. As saprophytes or parasites, they exhibit an almost ubiquitous condition. Bacterial groups recommended for use in agricultural production include non-pathogenic species

known as beneficial bacteria. Free-living microorganisms that promote plant growth, that are used as biological warfare agents or biological fertilizers are called rhizobacteria (PGPR) that promote plant growth.

These bacteria mostly belong to *Acetobacter, Acinetobacter, Achromobacter, Aereobacter, Agrobacterium, Alcaligenes, Artrobacter, Azospirillum, Azotobacter, Bacillus, Burkholderia, Clostridium, Enterobacter, Erwinia, Flavobacterium, Klebsiella, Microccocus, Pseudomonas, Pseudomonas, Rhizobium, Serratia and Xanthomonas* genus. They are the species that are functional by colonizing the area known as the rhizosphere in the plant root region and on the leaf surfaces of the plant called the phyllosphere.

The rhizosphere, which is chemically, physically and biologically affected by plant roots and is the soil region surrounding the roots, is a very suitable environment for the proliferation of microorganisms and has a potential on plant health and soil fertility. The rhizoplane in this region is the root surface of the plant that firmly holds the soil pieces (Çakmakçı 2005, Ertürk 2022). In the rhizosphere, important and intense interactions take place between plants, soil, microorganisms and soil microfauna. These interactions can affect plant growth and yield positively or negatively.

An important group of these microorganisms, which have beneficial effects on plant development on root colonization, was found by Kloepper and Scroth (1979) and named as plant growth-regulating rhizobacteria (PGPR).

PGPRs act directly on plant growth with plant growth regulators such as auxin, cytokinin and gibberellin, or by reducing ethylene in the plant, dissolving inorganic phosphate and mineralizing organic phosphate and nutrients, providing asymbiotic fixation of atmospheric nitrogen and stimulating disease resistance mechanisms (induced systemic resistance). It has indirect effects by acting as biocontrol agents that reduce diseases or by degrading xenobiotics in polluted soils by playing a role in protecting plants or promoting other beneficial symbiosis.

In addition, it also increases the tolerance of plants against stresses such as drought, high salinity, metal toxicity and pesticide excess (Bashan and de-Bashan 2014, Ertürk 2022) (Fig. 5).

It is well known that plant growth is regulated in part by hormonal changes, so changes in the hormonal content of the host plant may be necessary to stimulate plant growth by PGPR. For example, many bacterial species can produce more or less IAA. In addition, it has been reported to reduce the expression of auxin sensitive genes in plant roots (Lakshmanan et al. 2013). Also, another enzyme, ACC, is a precursor to ethylene, so lowering ACC levels will lower ethylene levels and inhibit the growth-reducing effect of this plant hormone. Some PGPR hydrolyze ACC to ammonia and α-ketobutyrate by the enzyme ACC deaminase and use the latter as a source of acarbon (Van de Poel and Van der Straeten, 2014). Some PGPR can reduce ABA levels in the host plant and subsequently indirectly increase plant growth (Belimov et al. 2014). The positive effects of PGPR are dependent on the endogenous ABA levels of the host plant. For example, under water stress conditions, some PGPR can increase the ABA content of host plants, thereby reducing water loss (Salomon et al. 2014).

Atmospheric nitrogen (N_2), which cannot be used by plants by biological nitrogen fixation (BNF) by some microorganism species, is converted into (NH_4) form with the help of nitrogenase enzyme.

With PGPR applications in horticultural cultivation, germination rate, root development, yield, leaf area, chlorophyll rate, nitrogen rate, protein rate, hydraulic activity, drought tolerance, root and stem weight increase, aging of leaves is delayed and resistance to some diseases is provided. PGPR applications are carried out in laboratory, greenhouse and field conditions, but unexpected conditions in field trials sometimes make it difficult to obtain appropriate results.

The emergence of unsuitable conditions such as pH changes in the soil, high temperature, low precipitation, humidity and nutrient deficiency reduces the colonization of microorganisms (Şahin et al., 2004; Dobbelaere et al. 2001). Preventing environmental pollution and agricultural sustainability, ensuring the continuity of resources, reducing agricultural costs, require the use of biological nitrogen fixers and phosphate solvent bacteria. PGPR is used for agriculture, horticulture, forestry and environmental regeneration. Apart from these functions, some bacteria like *Pseudomonas* synthesize hydrogen cyanide and inhibit the development of pathogenic fungi. It can prevent plant diseases caused by different fungi by hydrolyzing fusaric acid compounds that harm the

plant in *Fusarium* infection of species such as *Cladosporium werneckii*, *P. cepacia*.

In addition, as in *P. cepacia*, some bacteria are effective by damaging pathogenic fungal mycelia such as *Rhizoctonia*, *Sclerotium* and *Pythium*.

PGPRs can also protect plants from pathogens by competing for nutrients on the root surface. Inoculation of plants and seeds with PGPR can provide long-term systemic resistance to diseases. Antibiosis and competitive antagonistic interaction emerges between *Pseudomonas*-like bacteria and the target natural microflora (Ertürk 2022) (Fig. 6).

Applications with beneficial bacteria on annual horticultural plants have also been carried out in perennial plants in the last 10-15 years. The positive effects observed in annual plants were often not valid in many studies, and very different results were recorded according to ecology, plant and bacterial isolates in field conditions. Although many studies have been reported on these subjects, the physiological basis of the activities of beneficial bacteria still covers the issues that are waiting to be explained. Especially the histological and genetic basis of the effects of bacteria require further explanation.

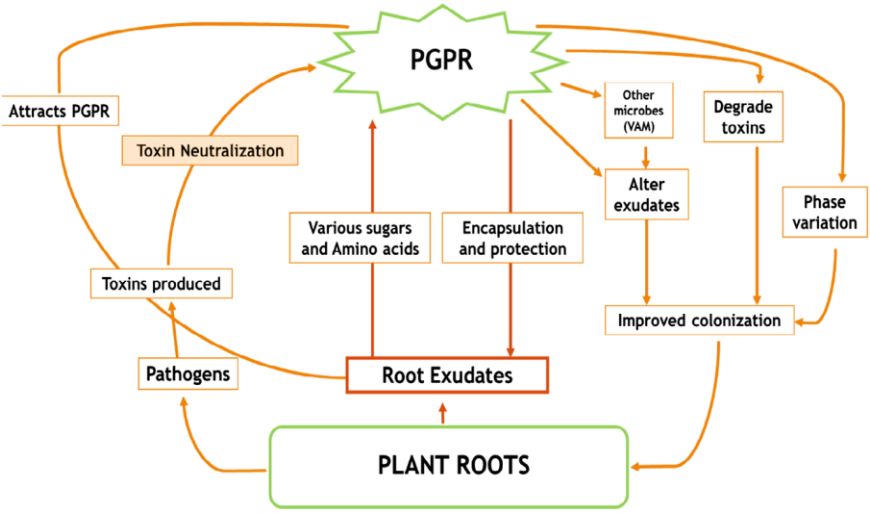

Figure 5. Interaction of plant root exudates, pathogens, PGPR, and other beneficial microbes in the rhizosphere (Dutta and Podile 2010).

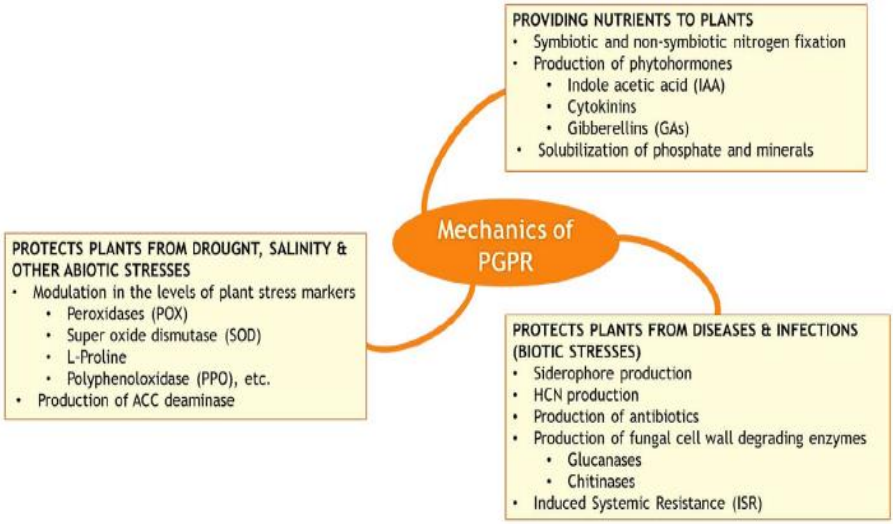

Figure 6. Schematic diagram representing mechanics of PGPR (Goswami et al. 2016)

2.7. Benefical Fungi

Modern horticulture has to achieve two conflicting goals, the need to produce food for the growing world population, and the need to minimize damage to the environment. For this process known as sustainability, many technological innovations have been the subject of research in recent years. Arbuscular mycorrhizal fungi have an important place among these study titles.

AMF occurs between roots and a particular group of fungi that are taxonomically distinguished from all other true fungi in the phylum *Glomeromycota* (Schüssler et al. 2001). It is stated that this fungal group is the most common plant symbiont and is present in 80-90% of land plant species (Newman and Reddell 1987). With a few exceptions of genera/species, all AMF can interact with all mycorrhizal plants. Therefore, it is not possible to recommend specific AMF species for certain horticultural crops. However, since species of the genera Gigaspora and *Scutelleospora* can be harmful to soil structure, most commercial vaccines contain species of the genera *Rhizophagus* and *Funneliformis*. These species are found in almost all soils with a wide range in all climatic zones and thus can be applied in horticultural production in all geographical regions (Smith and Read 2008).

As AMF, which plays an important role in plant performance and nutrition due to their capacity to improve plant mineral uptake, inoculants on the market, especially as phosphorus mobilizing products, are gaining even more importance. AMF symbiosis is particularly important for increasing the uptake of relatively inert and insoluble phosphate ions in soil due to interactions with divalent and trivalent cations, mainly Ca^{2+}, Fe^{3+} and Al^{3+} (Fitter et al. 2011). The basis of these functions is usually attributed to the capacity of AMF to develop an external hyphae network that can expand the surface area (up) (up to 40 times), as well as to the production of enzymes for nutrient uptake (such as the secretion of phosphatases to hydrolyze phosphate) and/or the excretion of organic matter (Marschner, 1998).

It has been reported with research results that AMF not only improves plant nutrition (biofertilizers), but also affects plant growth (bioregulators) and alleviates the effects of environmental stresses (bioprotector) by interfering with the plant's phytohormone balance (Kaya et al 2009, Khalil 2013, Wang et al 2014). , Xiao et al 2014, Boyer et al 2015, Balci et al 2021) (Fig 7). It has been reported that AMFs can induce changes in the secondary metabolism of plants that provide the biosynthesis of phytochemicals (secondary metabolites) that are beneficial to health (Rouphael et al 2015). In addition, AMF drought tolerance (Asrar et al 2012, Jayne and Quigley, 2014, Wang et al 2014), adverse soil conditions including salinity (Porcel et al, 2012, Gomez et al 2015, Balcı et al 2021), heavy metal pollution. (Prasad et al 2011, Kumar et al 2015) and unfavorable soil pH conditions (Cardarelli et al 2010, Seguel et al 2013, Rouphael et al 2015).

However, some factors such as the use of specific biocides, cultivation with non-host plants (such as *Brassicaceae*, *Chenopodiaceae*), high-level phosphorus fertilizer application, and some ecological factors with fungal genotype X plant species/varieties may adversely affect AMF symbiosis (Njeru et al. 2015, Rouphael et al. 2015).

The high quality inoculum required for successful root colonization with AMF should be in the form of mixtures of species known as AMF, contain a high number of infective AMF propagules, be free of plant pathogens and pests, have beneficial bacteria and additives that support

root mycorrhizal colonization and activity, and have a long shelf life if possible. It is very important that they are dry solid products with a lifespan. It is known that AMF propagules can be produced in the roots of plants grown outdoors or in containers in greenhouses (Feldmann and Schneider, 2008) (Fig 7).

AMF promoted the increase of ROS scavenging enzymes such as superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) and ascorbate peroxidase (APX) in the leaves of salt-affected plants. It has been reported that mycorrified plants can provide resistance as a result of lower lipid peroxidation accumulation, which shows lower oxidative damage (Rouphael et al 2015). Also, stomatal conductivity and net photosynthesis can be increased by AMF inoculation (Hajiboland et al 2010). AMF can be effective in reducing the detrimental effect of heavy metal contamination by immobilizing metals (Andrade and Silveria, 2008). It can be a barrier against the penetration of heavy metals (especially Cd, Pb and Ni) into the plant (Mozafar et al, 2002, Lee and George 2005, Andrade and Silveria, 2008, Kumar et al 2015).

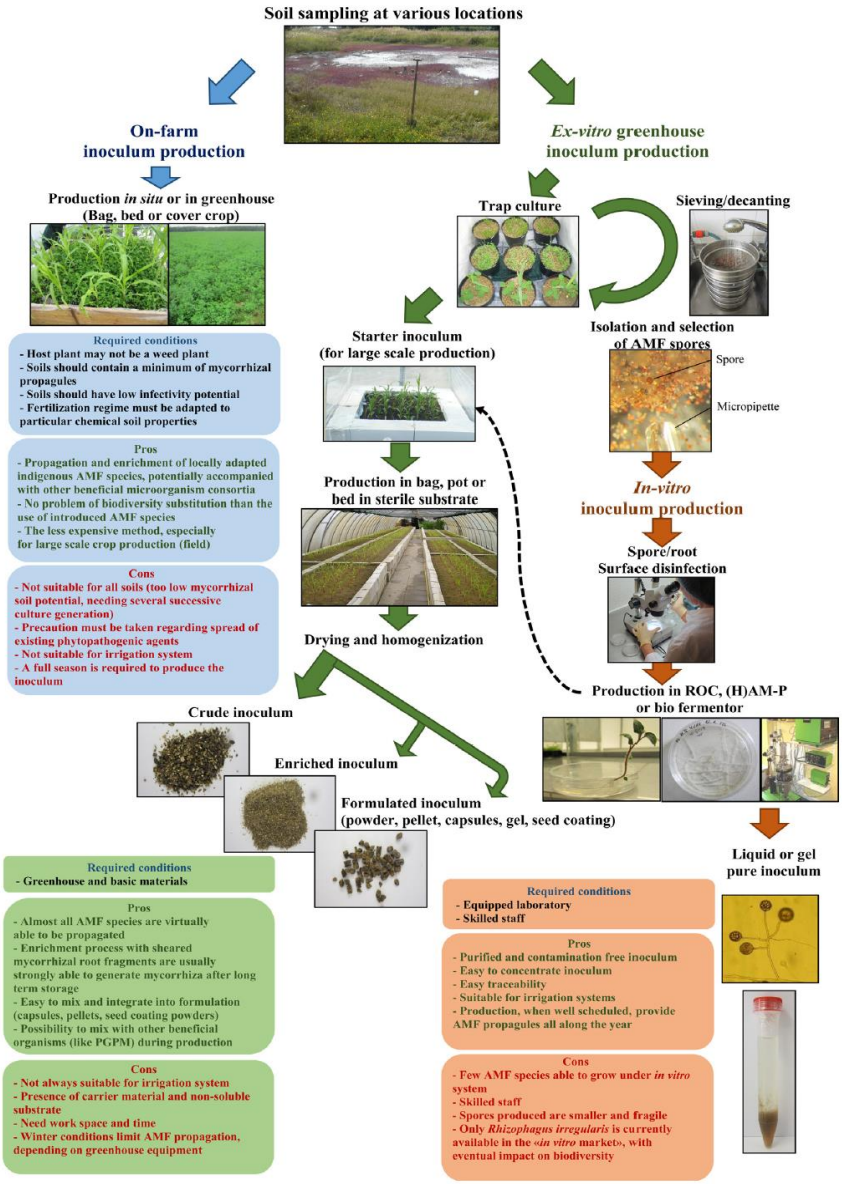

Figure 7. Arbuscular mycorrhizal fungal inocula can be produced on-farm, ex vitro in greenhouses or climate chambers or in vitro on plants, in root organ cultures (ROCs) orin biofermentors. Required conditions, advantages and disadvantages of the three technologies are summarised (Rouphael et al 2015).

CONCLUSION

In this study, in which the efficacy of bio-stimulators was evaluated, in summary, many issues such as the fact that most of the positive reports were made in controlled environments, some study results contradicting each other, and the organization of studies with these substances by considering many factors such as dose, plant species/variety, ecology will be constructed in this way. We think it would be more appropriate to prioritize studies. In addition, further studies on crop plants are needed to understand the mode of action of biostimulators and their interactions between environmental stressors and plant genotypes.

Revealing the activity mechanisms in the microbiota that determines the soil and plant interaction in histological and genetic context may also pave the way for more effective use of biostimulators together. Especially in the rhizosphere, the most accurate method of detecting the most effective microorganism composition and ensuring their effectiveness in field conditions is to determine the appropriate isolates in each ecology and plant species and to carry out the processes over these strains.

In the continuation of this process, it should not be forgotten that it is necessary for a sustainable agricultural production to include the best bio-promoting elements in the system for the continuity of the richest and most effective microbiota in the soil.

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CHAPTER 4

GENERAL SITUATION OF THE SEEDLING SECTOR IN TURKEY

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INTRODUCTION

World population and consumption are increasing year by year. Agricultural areas are decreasing for various reasons. For this reason, it is necessary to increase the yield from the unit area in order to produce sufficient foodstuffs. Efforts to increase yields have been carried out throughout the history of agriculture. With the advances in biology and genetics, plant breeding studies have accelerated and the targeted yield and quality have been tried to be achieved. Modern agricultural practices have also been one of the important factors that increase yields. In the world, 1.148.446.252 tons of vegetables are produced in a total area of 58.298.000 ha. China is the country that produces the most fresh vegetables in the world with a production of 596.166.271 tons. China has a 52% share of world fresh vegetable production. This country is followed by India (141.195.036 tons) and the USA (33.124.467 tons) respectively. Turkey ranks fourth in the world ranking with a production of 25.960.7414 tons and receives a share of 2.2% of world fresh vegetable production. While world vegetable production increased by 5.5% from 2016 to 2020, this rate is approximately 6.3% in Turkey (Table 1).

Table 1. Important world fresh vegetable producing countries by year

Countries	Years				
	2016	2017	2018	2019	2020
China	559.740.272	576.155.633	581.137.843	587.222.613	596.166.271
India	125.447.083	131.334.057	135.590.864	137.401.659	141.195.036
USA	34.135.076	32.078.987	31.732.094	32.983.409	33.124.467
Turkey	24.421.408	24.923.427	24.172.654	25.382.178	25.960.714
Egypt	15.256.770	15.516.968	15.384.986	16.006.802	16.135.024
Iran	13.391.399	10.831.814	10.631.549	11.473.559	12.623.192
Italy	13.046.726	12.487.354	10.351.730	10.436.580	10.849.360
Mexico	14.249.718	15.434.608	16.086.696	15.131.963	15.098.212
Nigeria	14.123.702	14.595.571	15.435.352	15.778.612	15.706.483
Russia	13.190.558	13.628.739	13.716.321	14.152.745	13.950.679
Viet Nam	15.192.692	15.717.386	16.263.792	16.670.940	17.002.195
Ukraine	9.498.005	9.304.222	9.458.376	9.706.738	9.675.389
World	1.087.828.966	1.109.345.242	1.111.045.830	1.129.672.958	1.148.446.252

(Fao, 2022)

The most produced vegetable in Turkey is tomato with 13.204.015 tons and its share in world tomato production is at the level of 7%. The second most produced vegetable in Turkey is watermelon with 3.491.554 tons and its share in world production is 3%. The Turkish production of pepper, which has a share of about 7% in world production, is 2.636.905 tons. The other most produced vegetables in Turkey are dry onion (2.280.000 tons), cucumber (1.926.883 tons), melon (1.724.856 tons), cabbage (851.648 tons) and eggplant (835.422 tons) respectively (Table 2).

Table 2. Important vegetables produced in the world and Turkey

Vegetables	Turkey	World	%
Tomato	13.204.015	186.821.216	7.07
Watermelon	3.491.554	101.620.420	3.44
Pepper (green)	2.636.905	36.136.996	7.30
Onion (dry)	2.280.000	104.554.458	2.18
Cucumber	1.926.883	91.258.272	2.11
Melon	1.724.856	28.467.920	6.06
Cabbage	851.648	70.862.165	1.20
Eggplant	835.422	56.618.843	1.48

(Fao, 2022)

While vegetable production is carried out in 7.553.346 decares area in Turkey, the agricultural area under cover is 854.599 decares. Another condition for obtaining healthy, high quality and efficient products in crop production is to grow healthy seedlings and plants. While the number of seedlings used in vegetable production in Turkey in 2012 was 3 billion, this number was 5.5 billion in 2021. In 2021, a total of 1.096.715.981 vegetable seedlings were produced in Antalya (Figure 1). With 506.776.834 units, tomatoes constitute approximately 46.2% of the total seedling production, while peppers account for approximately 20.8% with 228.609.384 units. These two vegetable seedlings are followed by cucumber (9.2%), lettuce (8.6) and watermelon (5.4%), respectively (Table 3).

Table 3. Production Amounts of Seedling in Antalya

Vegetables	Number of seedlings	%
Tomato	506.776.834	46.2
Pepper	228.609.384	20.8
Cucumber	101.926.726	9.2
Eggplant	44.324.825	4.0
Watermelon	59.482.049	5.4
Melon	33.681.636	3.0
Pumpkin	8.907.336	0.8
Cabbage	18.415.331	1.7
Lettuce	94.591.860	8.6
Total	1.096.715.981	

(Fidebirlik, 2021)

Advantages of Production with Seedlings

- Earlyness
- Protection of summer vegetable species from low temperature risks in the spring period
- To be free from disease pests;
- Obtaining homogeneous seedlings,
- Prevention of seed loss,
- Energy saving,
- Greater adaptability compared to seed;
- Production with less labor than seed production
- Prevention of irregular germination and growth caused by seed production

Figure 1. Turkey seedling production between 2012 and 2021 (TÜRKTOB, 2021)

In order to grow healthy seedlings and plants, biotic and abiotic factors should be given importance to factors such as genotype, fertilization, growing conditions of the mother plant, the maturity period of the seed during harvest, seed processing techniques, seed moisture content during storage and seed quality, as well as strong and dense root structures, they receive more water and nutrients from the soil depending on the increase in the number of absorbent feathers, length and root length and can transmit upper disruption; Due to the excess amount of hormones synthesized in the roots, plant growth should be increased (Fernández and Martínez, 2002; Kovalev, 1990; Ra et al. 1995; Ruiz and Romero, 1999). This can only be achieved either with plants with strong roots or with the use of seedlings grafted on strong rootstocks. Grafting is carried out in vegetables for purposes such as combating soil-borne diseases (Balázs et all. 2011; Edelstein et all, 2011), more efficient intake and use of water and nutrients (Robinson and Decker-Walters, 1997), stronger development of plants (Lee, 1994; Lee and Oda, 2010), growing double crops by grafting tomatoes and eggplants on potatoes (Lee, 1994), protecting the environment by reducing the use of pesticides with the use of disease- and pest-resistant/tolerant rootstocks (Lee, 1994; Lee and Oda, 2010) and increasing earlyness and yield by developing the plant strongly in the early period (Ruiz and Romero, 1997). In Antalya province, 206.029.353 grafted seedlings were produced in 2020 in grafted species, which is 19.6% of the total number of seedlings. The highest rate of grafted seedlings was in watermelon plants with 79.9%, followed by eggplant (45.4%), tomatoes (18.4%), melons (11.4%) and

cucumbers (8.1%). In 2020, grafted pepper seedling production (0.0%) was not recorded (Table 4).

Table 4. Production Amounts of Grafted Seedling in Antalya

Vegetables	Grafted	Non-grafted	Total	%
Tomato	121.123.429	536.314.132	657.437.561	18.4
Pepper	0.0	163.592.579	163.592.579	0.0
Cucumber	7.476.058	84.374.898	91.850.956	8.1
Eggplant	21.620.012	25.989.263	47.609.275	45.4
Watermelon	52.736.232	13.298.961	66.035.193	79.9
Melon	3.073.622	23.988.164	27.061.786	11.4
Total	206.029.353	847.557.997	1.053.587.350	19.6

(Fidebirlik, 2021)

In 2008, with 41 members, Seedling Producer Organizations gathered under the roof of Fidebirlik. The number of members of Fidebirlik reached 58 in 2009, 70 in 2010, 75 in 2011, 87 in 2012, 90 in 2013, 94 in 2014, 108 in 2015, 111 in 2016, 122 in 2017, 148 in 2018, 151 in 2019, 164 in 2020 and 190 in 2021. As of the end of October 2022, the Association has 212 members. Of these 212 members, 181 produce vegetables, 28 produce strawberries, 2 produce tissue culture, and 1 produces medicinal aromatic plant seedlings (Fidebirlik, 2021).

PROBLEMS AND SOLUTION OFFERS OF SEEDLING CULTIVATION SECTOR

Companies That Do Not Have a Producer Certificate and Produce Unregistered Seedlings

Since such companies carry out production without being subject to inspection, it also negatively affects the healthy development of the sector. They are businesses that undermine trust in seedling companies, create unfair competition, cause unregistered and illegal production, and do not have a producer's permit. These companies also cause the spread of diseases and pests that can be spread by seedlings. In order for the sector to be registered, the need for the Ministry of Agriculture and Forestry to establish an Agricultural Information System for seedling production is reported by sector representatives.

Seed Borne Disease Problems

According to the current legislation, the disease analysis of imported vegetable seeds has to be done in quarantine laboratories. Despite this, enterprises using imported vegetable seeds may also see seed-borne disease problems. There is no such quarantine requirement for domestic seeds produced in Turkey that are not imported. Therefore, businesses that grow seedlings using domestic seeds take on much more risk. Emerging seed-borne seedling diseases cause significant financial losses for both seedling enterprises and farmers, and cause legal problems by bringing the parties against each other. Establishment of an accredited laboratory in accordance with international norms, which can make rapid disease diagnosis in domestic or foreign sourced vegetable seeds, will be effective in solving the problem. In this regard, the initiative to establish an accredited diagnostic laboratory in cooperation with Fidebirlik, the Ministry and the Turkish Seed Association is a promising development.

Seed Germination Problems

The numerical data obtained from the germination tests performed by the seed companies are based on the test results made under laboratory conditions. However, these results sometimes may not comply with the conditions and conditions in the seedling production facilities. The test results, which do not take into account the germination power of the seeds, do not reflect the actual performance of the seed. Seeds with weak germination power germinate late, and seedlings that develop from these seeds show a weak development by staying in the shadow of early germinating seedlings because they are smaller. In modern seedling enterprises, even though homogeneous depth of sowing is done in automatic sowing machines and germination is carried out in controlled conditions (humidity-temperature-light) in germination chambers, significant differences may occur in some lots in terms of emergence times. It is not possible to present a seedling of homogeneous size and quality to the farmer with the gradual germination that occurs in very different day intervals in the seedling enterprises in the same lot. This late germination also negatively affects seedling production and shipment planning. This important problem needs to be resolved quickly between the international seed and seedling sectors.

The Fluctuation in the Exchange Rate Increases the Input Costs

One of the most important factors affecting the input costs of seedling enterprises is that the input is supplied from the country or abroad. Most of the hybrid vegetable seeds, especially the plant species used in greenhouse cultivation, originate from abroad and are priced as indexed to foreign currency. The upward movement in exchange rates, especially in the last two years, causes the prices of imported hybrid seeds to increase significantly. The increase in the exchange rate not only increased the seed prices, but also the main inputs of the seedling enterprises; It also significantly increases the prices of products whose raw materials are supplied from abroad, such as peat, vermiculite, chemical pesticides and fertilizers, viols, inserts, silicone graft clips, plastic cases, cardboard boxes. The increase in the exchange rate increases the transportation costs by increasing the fuel prices; The increase in energy input costs by raising the prices of coal, natural gas and electricity also negatively affects the seedling enterprises. Despite the high employment it provides, the fact that seedling enterprises are not accepted as industrial establishments causes the industry to not benefit from the advantage of cheap energy provided. The sector, which needs electrical energy intensely in order to operate systems such as irrigation, fertilization, ventilation, lighting and automation, can use this energy at a lower cost, which will benefit the sector and therefore the farmer.

Inadequacy of Rootstock Breeding Studies in Grafted Seedling Production

Grafted seedling production, which was approximately 129 million units in 2019 in Antalya province, was realized as approximately 206 million units in 2020. Grafted seedlings will be more preferred all over the world in the coming years due to the tolerance they provide against biotic and abiotic stress factors. With these superior features compared to ungrafted seedlings, grafted seedlings provide ease of application in sustainable agriculture methods such as organic agriculture and good agricultural practices, and in integrated pest management (IPM). Although the demand for grafted seedlings is mostly concentrated in watermelon, eggplant, tomato, cucumber, melon and pepper types, it is predicted that grafted seedlings will gain more importance in other

vegetable types in the ongoing process. For this purpose, it has emerged that it is necessary to focus on the diversity and breeding of rootstocks to be used in grafting, and studies on this subject have gained momentum.

Chemicals Used to Provide Height Control in Seedlings

Another important quality criterion sought in vegetable seedlings is that the seedlings have a balanced root body development rate. Light competition between seedlings after frequent planting, use of small viol; Due to various reasons such as excessive irrigation, rapid growth as a result of fertilization, excessive grading may occur in the seedlings. Seedlings with thin trunk diameter, long internodes, and over-sorted seedlings are not preferred by the producers due to their low field performance after planting. Seedling enterprises generally experience the problem of over-sorting due to lack of lighting in the spring and rapid growth in the fall. Although there are many different applications to prevent this, they prefer chemical inhibitors that act as gibberellic acid inhibitors in terms of both high efficiency and practicality. However, the most widely used drugs with active ingredient Paclobutrazol are licensed for some fruit trees and ornamental plants, but not for vegetable species. In 2020, the product with the trade name Cultar 25 SC (250 g/L Paclobutrazol) was licensed only for tomato. Many of the seedling enterprises use such chemicals to provide height control in many types of vegetables, especially cucurbits. However, they cannot declare the application due to the license problem. In order to reduce the risks of such chemical inhibitors, which have a long half-life, on human health and the environment, it is of great importance to investigate especially organic-based inhibitors.

The Problem of Not Finding Qualified Personnel

Seedling cultivation is a technical business that requires expertise. Especially nowadays, when grafted seedlings are widespread, it requires a higher level of expertise. Therefore, there is a need for technical personnel (Agricultural Engineer, Technician, Technician) who have received academic training on this subject. Unfortunately, in most of the faculties of agriculture in our country, the "Seedling and sapling production" course is an elective course, and many students graduate without taking this course. Seedling cultivation is generally explained in the "Greenhouse Cultivation" course for 1-2 weeks. This situation makes it difficult to find personnel with technical competence. The

sector has to train the personnel it employs. In order to contribute to the solution of the problem, it would be beneficial to give such courses as compulsory in the faculties of agriculture.

Classification of Businesses in the Sector

The Ministry of Agriculture and Forestry has prepared a draft regulation on the equipment that the seedling producer should have. According to this regulation, seedling producers are required to be defined separately as A and B classes. In order to increase the success of the seedling sector; production should be done in modern greenhouses using advanced technological equipment. Otherwise, unregistered and poor quality production, which the industry has been struggling with for a long time, will increase.

Insufficient Agricultural Supports

Low-interest loans are provided in the establishment phase and operation process of modern seedling enterprises. However, there is no government support given to our farmers who supply seedlings from modern ready-made seedling enterprises. The Ministry provides support only for certified strawberry seedlings, not vegetable seedlings. State support should be given to the farmer who buys the vegetable seedlings produced using certified seeds from seedling enterprises with seedling production permit. In this way, informality in the seedling sector can be prevented and farmers are supported. In order to encourage and increase the use of grafted seedlings, support should be given to farmers using grafted seedlings at higher numbers.

Problems with the Use of Seasonal Workers

Seedling businesses, which provide employment opportunities with a short-term training, especially women's employment, contribute to the economy of the region and country where they are located. In the current Labor Law, agricultural enterprises employing up to 50 workers are exempted from notice and severance pay when seasonal workers leave at the end of the season. The number of workers that seedling enterprises have to employ in some periods exceeds 50. For example, this problem is more common in enterprises that produce grafted seedlings, as more workers are needed during the grafting season. When the number of workers in enterprises exceeds 50, they lose their right to notice and severance pay exemption. As a result, this practice means

penalizing businesses that create employment and encourages the employment of unregistered uninsured workers. Therefore, it will be beneficial to increase the exemption limit in order to support the enterprises whose number of enterprises and seedling production capacity are increasing day by day and to prevent unregistered workers.

Problems Caused by VAT Applications

In Article 28 of the Value Added Tax Law numbered 3065, it is stated that 8% value added tax should be applied for seeds, seedlings, saplings, steels, mycelium, spores and the like used for growing fresh vegetables and fruits in section A/5 of the attached list no II. . However, in practice, seedling companies apply 8% VAT on their own seed and seedling sales, and 18% VAT on the farmer's seed, considering it to grow seedlings. However, the annex of the Presidential Decision No. 5189, published in the Official Gazette dated 13 February 2022 and started to be implemented as of 14 February 2022, "The Decision on the Amendment of the Decision on the Determination of the Value Added Tax Rates to be Applied to Goods and Services" has re-introduced the VAT rates applicable to Foodstuffs for which SCT is not applied. has arranged. On the positive side of the decision, it is stated that the VAT rate to be applied on vegetable and strawberry seedlings in Article 6 of the Foodstuffs section has been reduced from 8% to 1%. However, it has also been reported that the VAT rate applied to the seed growing fee will continue as 18%. Seedling production is not a service provided by contract production, but a real production in its full sense. For this reason, a single VAT rate should be applied for seedling producing enterprises. As non-company farmers do not have the opportunity to deduct the 18% VAT burden they have to pay when they procure the seeds themselves, their burden increases even more.

Status Problem of Seedling Enterprises

As seedling enterprises are not considered as a manufacturing industry, as in other agricultural establishments, they are not taken into SME status. Enterprises producing seedlings, which is one of the most important inputs for agriculture; they cannot benefit from the support provided to SMEs such as fair participation, employee training, low interest loan support, energy support. Making seedling businesses benefit from the rights granted to SMEs will contribute significantly to the development of the sector. It would be beneficial

for seedling production facilities to be accepted as industrial facilities and included in the scope of SMEs.

Seedling Inspection Problem Made by the Provincial Directorates of the Ministry of Agriculture and Forestry

According to the current regulation, it is obligatory for the seedling enterprises to pay the inspection fees and obtain documents for each seedling shipment to the provincial directorates. It is not possible for the Provincial Directorates, which have a limited number of inspection personnel, to bear this burden, especially in the provinces where there are many seedling enterprises, especially in Antalya, where seedling enterprises are concentrated, and especially during the periods when seedling shipments are intense. Shipments are disrupted, and farmers and seedling companies may come face to face. The increase in the number of seedling enterprises and the increase in their production capacity show that the current system is unoperable. In the countries where the sector is developed, the method of licensing the enterprises and performing the routine inspection procedures is applied as the inspection system. Seedling enterprises pay the license and inspection fee once a year, and they intervene during the inspection if there is a deficiency arising from the system and production.

CONCLUSION

With its 790 thousand hectares of vegetable production area, Turkey has the potential to use more than 15 billion seedlings. Considering that the current vegetable production amount is around 5.5 billion units, it is understood that the sector has a great potential. In addition, seedling production for organic seedlings, medicinal and aromatic plants and the increase in seedling demands of hobby gardeners will further enlarge the seedling sector. Due to the increasing demand for grafted vegetable seedlings day by day, it is necessary to support the use of grafting machine or robot, which provides significant advantages in terms of time, labor and cost.

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CHAPTER 5

COMPARISON OF YIELD AND YIELD PARAMETERS OF LINSEED (*LINUM USITATISSIMUM* L.) CULTIVARS AT DIFFERENT SOWING NORMS

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INTRODUCTION

Anatolia is one of the origin centers of linseed. Linseed culture has been cultivated in Anatolia for thousands of years. It is generally planted for its seed in Central Anatolia and is known by the names 'Zeğrek' or 'Zeyrek'. It is also called as linseed oil in industrial areas (Ertuğ, 1998). Linseed oil is mostly known for its industrial use, and it has been used as oil lamp for lighting in Anatolia, as fodder and therapeutic oil in livestock, and as cooking oil in kitchens until recently. This extraordinary plant has been used as a cough suppressant, wound healer and pain reliever in folk medicine (Baytop, 1984, Ertuğ, 1998). However, there has been a decrease in the cultivation area and production of linseed each year.

There are annual and perennial types of flax plant. One of these species, Linum usitatissimum, is herbaceous annual and growing period is completed within 3 to 4 months from planting. When densely planted for fibre, plants average 80 to 120 cm in height, with slender stalks 2.5 to 4 mm in diameter and with branches concentrated at the top. Plants cultivated for seed are shorter and many-branched. The leaves, alternating on the stalk, are small and lance-shaped. The flowers, borne on stems growing from the branch tips, have five petals, usually blue in colour but sometimes white or pink. The fruits are small dry capsules composed of five lobes and 4 to 10 seeds in a capsule. Thousand seed weight can vary between 4 g and 15 g. The shape of the seeds resembles sesame seeds, but the difference is that their surfaces are shiny, slippery and the ends are beaked (Anonymous, 2022, Baydar ve Erbaş, 2014, Zuk ve diğ., 2015).The plant is adaptable to a variety of soils and climates but grows best in well-drained sandy loam and in temperate climates. In most areas planting of the same land with flax is limited to once in six years to avoid soil exhaustion. Cool moist growing seasons produce the most-desirable fibre (Anonymous, 2022).

In parallel with the rapid population growth in the world, the need for foodstuffs is increasing. In order to meet this need, it is necessary to increase the products obtained from agricultural areas. This will be possible by increasing the yield per unit area for agricultural production or by increasing the production areas. New agricultural areas Unfortunately, the use of these areas for agricultural production is decreasing or being lost due to the expropriation of first-class agricultural lands for urbanization and industry or

the erosion and landslides increasing due to the destruction of nature. The decrease or the inability to increase the agricultural areas due to these and similar reasons makes it necessary to increase the yield to be obtained from the unit area in order to meet the increasing food demand in the world (Demir, 2009).

Linseed, which can be grown for the purpose of oil and fiber, which can be used both in the industrial field and in traditional treatment methods, makes the linseed plant advantageous because it has summer and winter forms and can be included in alternation systems. (Endes, 2010; Kurt et al., 2015). Our country has a significant oil deficit and in order to close this deficit, it is needed to grow alternative oilseed plants such as linseed plant.

MATERIAL AND METHODS

This study, which aims to determine the effects of different sowing norms on yield and yield elements of oil linseed cultivars, was carried out in Kırşehir Ahi Evran University Faculty of Agriculture Research and Application Area in 2021. The trial area is located at an altitude of 1014 meters above sea level, at 39.15° North latitude and 34.11° East longitude.

Table 1. Soil characteristics of the research erea

Soil parameter	0-30 cm depth	30-60 cm depth
Saturation (%)	55	55
pH	7.59	7.63
Soil EC (mmhos/cm)	0.52	0.56
$CaCO_3$ %	27.9	28.39
Phosphor (P_2O_5 kg/da)	2.14	2.29
Organic Matter (%)	1.81	1.64
Potassium (K_2O kg/da)	66.62	51.47
Total salt (%)	0.02	0.02

Experimental land soil is classified as clayey-loamy textured, alkaline, calcareous, unsalted, with a certain amount of usable phosphorus concentration, rich in potassium, poor in nitrogen and organic matter (Table 1) (Kacar, 1994).

According to the monthly total precipitation heavy precipitation was observed especially in March 2021, and it was observed that long term precipitation especially in April and May were significantly higher than 2021.

While the total precipitation of linseed growth period from March to the end of July was 165.4 mm in 2021, this value was calculated as 185 mm in long term total monthly precipitation. It is seen that 2021 has drier weather conditions in terms of monthly long term air humidity mean and 2021 was 5.4% lower than the average of March-August of long term (Table 2).

Table 2. Climate data of research area

	Total Precipitation		**Temperature (°C)**		**Humidity (%)**	
Months	**2021**	**1991-2021**	**2021**	**1991-2021**	**2021**	**1991-2021**
March	95.2	37.9	4.5	5.9	65.5	66.7
April	19.4	42.7	12.0	10.8	56.5	62.7
May	9.2	46.2	18.2	15.7	45.3	60.6
June	35.1	37.5	19.3	20.0	55.1	54.9
July	0.9	8.9	24.9	23.7	40.4	46.9
August	5.6	11.8	24.3	23.9	43.4	46.6
Total/ Average	165.4	185.0	17.2	16.7	51.0	56.4

The experiment was set up in a randomized block split plot design with 3 replications. Linseed cultivars (Sarıgelin, Karakız, and Beyazgelin) were placed in the main plots and sowing norms (200, 400, 600, 800, 1000 and 1200 seeds/m²) were placed in the sub plots. Each sub-plot of the experiment was planted on March 21, 2021, with 25 cm row spacing and 4 rows of 5 m length. According to the results of soil analysis, 80 kg/ha of pure N was used for all plots. Half of the nitrogen was applied as base fertilizer (20.20.0 fertilizer) with planting, and the other half was applied as top fertilizer during hoeing and dilution (33% AN form). All plots were given 6 kg/da P_2O_5 by using 20.20.0 fertilizer and triple super phosphate (TSP) fertilizer as phosphorus source at planting time. As maintenance operations, hoeing was done when the plants were 3-4 true leaves and before flowering and weed control was provided. Harvesting was done by hand from 17 July to 1 August after full maturity.

Plant height (cm): It will be calculated by measuring the main stems of randomly selected plants from each sub-plot before harvest. Starting from the soil surface, the length between the top capsule (up to the apex) will be measured and averaged.

Number of capsules per plant (pieces): Before harvest, the capsules on the plants in each sub-plot will be counted and calculated by taking the average.

Number of seeds per capsule (pieces): The number of seeds for a total of 30 capsules, 3 capsules representing the plant from each of the 10 randomly selected plants, will be counted and calculated by taking the average.

Capsule width (mm): A total of 30 capsules, 3 capsules representing the plant from each of the 10 randomly selected plants, will be measured and averaged from the 1/3 height and thickest part with a caliper device.

Capsule length (mm): The length of a total of 30 capsules, 3 capsules representing the plant from each of the 10 randomly selected plants, will be measured with a caliper device and the average will be taken.

Thousand seed weight (g): In the seed samples taken, groups of 4x100 will be counted manually, weighed on a 0.01 g precision scale, and the average will be calculated by multiplying by 10.

Crude oil rate (%): After taking a certain amount of seeds from each plot and grinding with the help of a grinder, 3 g of the samples obtained will be weighed on a precision scale and analysed with petroleum ether in an automatic oil determination device, and the obtained values will be given in %.

Seed yield (kg/da): After the seeds taken from each plot were threshed separately, they were cleaned and weighed, and these values will be calculated by converting them to kg/da over the unit area.

Crude oil yield (kg/da): Crude oil yields per hectare will be calculated by multiplying the seed yields per decare with the crude oil ratio of the same plot (%).

Evaluation of Trial Results

The data obtained in the experiment were subjected to variance analysis in the MSTAT-C package program (Russell, 1986). The differences between the applications were evaluated by grouping them according to the Duncan comparison test (Düzgüneş et al., 1987).

RESULTS AND DSICUSSION

Plant height

In the study, it was found that the change in average plant height was different between varieties and this difference was statistically significant at the level of 1% compared to cultivars (Table 3). Among the linseed varieties, the longest average plant height of 34.71 cm belonged to the Karakız variety, while the shortest plant height average was 31.27 cm from the Beyazgelin variety (Table 3). Reddy et al., (2013) reported that although it was reported that the

heritability of plant height in linseed plants was high and it was mainly influenced by genotype, environmental factors were also effective. While there were differences between varieties in our study, the fact that the effect of sowing norm on plant height was not statistically significant can be attributed to environmental conditions, especially the low amount of precipitation during the development period of linseed plant. In similar studies with linseed plants (Chauhan et al. 2008, Ghanbari-ovi et al. 2013, Muhammad Bismillah Khan et al. 2005) it was reported that the plant height ranged from 55 to 88 cm, but the plant height obtained in our study was quite low. In this study, which we carried out in dry farming conditions, low rainfall and temperature increase in the growing season caused a significant decrease in plant height.

Number of capsules

In the study, it was determined that the number of capsules differed according to the cultivars and sowing norm, and this difference was statistically significant at the level of 1% (Table 3). While the average number of capsules was 4.83, the highest number of capsules among the linseed cultivars belonged to the Sarıgelin cultivar with an average of 5.32, while the least number of capsules was from the Karakız cultivar with 4.03 (Table 4.14). According to the seeding norm, the number of capsules varied between 3.06 and 6.66 pieces, and the highest number of capsules was obtained 6.66 pieces from 200 seeds/m² sowing norm, while the lowest number of capsules was observed 3.06 pieces from 1200 seeds/m² seeding norm.

Our findings regarding the number of capsules were lower than reported research, Diri (1996), Kurt (1996b), Siddique et al. (2002), Yıldırım (2005), Kurt et al. (2006) and Tunçtürk (2007) reported 17.3-27.1, 20-35.6, 9.2-13.9, 24.8-37.5, 16.1-37.2 and 14.2-25.6 capsules, respectively. The number of capsules in linseed may vary depending on agricultural practices and climatic conditions, apart from genetic characteristics (Yıldırım (2005) and Bozkurt and Kurt (2007)).

Number of seed in capsule

In the experiment, it was determined that the number of grains in the capsule of linseed cultivars differed according to the repetitions, varieties and sowing norm, and this difference was statistically significant at the level of 5%

between the replications and at the level of 1% according to the varieties and sowing norm (Table 3). While the average number of seeds in the capsule was 7.23, the highest number of seeds was 7.95 pieces from Beyazgelin cultivar, and the least number of seeds was obtained from Sarıgelin cultivar with 6.80 pieces (Table 3). According to the sowing norm, the number of seeds varied between 6.66 and 7.95, and the maximum number of seeds was obtained from the average of 200 seeds/m² sowing norm, while the minimum number of seeds was observed from the sowing norm of 1200 / m². The results of our research in terms of the number of seeds in the capsule has similarity with previous research, Diepenborck and Iwerson (1989) 7.8, Casa et al. (1999) 4-7, Siddique et al. (2002) 6-7 pieces, D'Antuono and Rossini (2006) 4.1-8 pieces.

Table 3. Variance analysis results and means of plant height, number of capsules, number of seed in capsule.

		Plant height (cm)	Number of capsules	Number of seed in capsule
Replication	2	2.224ns	1.34ns	0.714*
Cultivar (A)	2	57.896**	8.721**	7.064**
Error1	4	2.554	0.324	0.072
Sowing rate	5	3.743ns	14.539**	1.62**
AxB	10	6.517ns	0.807ns	0.505ns
Error	30	3.375	0.509	0.263
Cultivars				
Karakız		34.71A	4.03B	6.94B
Beyazgelin		31.27B	5.13A	7.95A
Sarıgelin		32.12B	5.32A	6.80B
Sowing rates (seeds/m^2)				
200		32.46	6.66A	7.95A
400		32.27	5.71B	7.14B
600		32.81	5.03BC	7.37AB
800		33.37	4.53CD	7.07B
1000		33.47	4.00D	7.16B
1200		31.82	3.06E	6.66B
Cultivars x Sowing rates (seeds/m^2)				
Karakız	200	33.4	5.66	7.83
	400	33.53	4.8	6.63
	600	34.3	3.83	6.96
	800	35.5	3.23	6.56
	1000	35.23	3.43	7.43
	1200	36.33	3.26	6.23
Beyazgelin	200	32.96	7.13	8.63
	400	31.73	5.9	7.53
	600	31.86	5.26	8.43

	800	31.9	5.23	7.7
	1000	31.26	4.46	7.53
	1200	27.9	2.83	7.86
Sarıgelin	200	31.03	7.2	7.4
	400	31.56	6.43	7.26
	600	32.26	6	6.73
	800	32.73	5.13	6.96
	1000	33.93	4.1	6.53
	1200	31.23	3.1	5.9

Capsule width

The changes in the capsule width of the linseed were significant at the level of 1% according to the sowing norm and significant at the level of 5% according to the interaction of the cultivar and sowing norm (Table 4). The average capsule width was 7.22 mm. According to the seeding norm, the longest capsule width was 7.40 mm from the 200 seeds/m² sowing norm, and the lowest capsule width was 7.10 mm from 1200 seeds/m² sowing norm (Table 4).

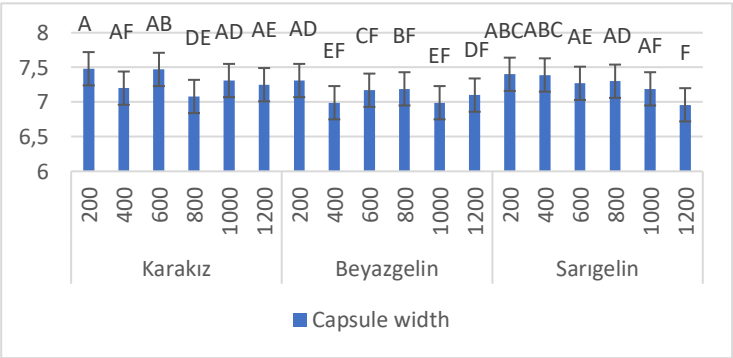

Figure 1. Capsule width changes for cultivar and sowing rates of linseed cultivars.

The interaction of cultivar and sowing norm, the highest capsule width was 7.48 mm in 200 seeds/m² seed amount of Karakız variety. The lowest capsule width was 6.96 mm, from the sowing norm of 1200 seeds/m² of Sarigelin variety (Figure 1). It is clear that increase in sowing norm resulted with decrease in capsule width Our research findings are similar to the values of 6.4-7.7 mm determined by Endes (2010), who studied the variety and sowing time of linseed.

Capsule length

In the study, it was determined that the change in the capsule length of the linseed was statistically significant at the level of 5% to the cultivars (Table 4). While the average capsule length in the study was 8.49, the longest average capsule length was 8.67 mm in Karakız, while the shortest average capsule length was 8.36 mm in Sarıgelin variety (Table 4). Our findings of capsule length values was changed beteen 8.20 and 8.86 mm and this results was similar with results of 6.1-8.8 mm for Yıldırım (1998), 7.6-10.5 mm for Akçalı Can (1999), 6.5-8.0 mm for Yıldırım (2005), and Endes (2010) 7.1-8.0 mm values.

Table 4. Variance analysis results and means of capsule width, capsule length, thousand seed weight.

		Capsule width (mm)	Capsule length (mm)	Thousand seed weight (g)
Replication	2	0öd	0.023öd	0.017öd
Cultivar (A)	2	0.143öd	0.465*	4.33**
Error1	4	0.032	0.049	0.038
Sowing rate (B)	5	0.102**	0.037öd	0.508**
AxB	10	0.049*	0.042öd	0.01öd
Error	30	0.022	0.027	0.041
Cultivars				
Karakız		7.29	8.67A	7.41A
Beyazgelin		7.12	8.46B	6.53B
Sarıgelin		7.25	8.36B	6.60B
Sowing rates (seeds/m^2)				
200		7.40A	8.61	7.24A
400		7.19B	8.45	6.99AB
600		7.30AB	8.52	6.86BC
800		7.19B	8.43	6.70C
1000		7.16B	8.48	6.66C
1200		7.10B	8.47	6.63C
Cultivars x Sowing rates (seeds/m^2)				
Karakız	200	7.48A	8.86	7.83
	400	7.20AF	8.67	7.57
	600	7.47AB	8.82	7.47
	800	7.08DEF	8.45	7.23
	1000	7.31AD	8.54	7.2
	1200	7.25AE	8.68	7.17
Beyazgelin	200	7.31AD	8.49	6.83
	400	6.99EF	8.36	6.73
	600	7.17CF	8.4	6.5
	800	7.19BF	8.42	6.4
	1000	6.99EF	8.56	6.37

	1200	7.10DEF	8.54	6.33
Sarıgelin	200	7.40ABC	8.48	7.07
	400	7.39ABC	8.34	6.67
	600	7.27AE	8.35	6.6
	800	7.30AD	8.42	6.47
	1000	7.19AF	8.34	6.4
	1200	6.96F	8.2	6.4

Thousand seed weight

It was determined that the change in thousand seed weight was significant at the level of 1% according to the cultivar and sowing norm, while it was determined that the thousand seed weight was not important according to the interaction of cultivar x sowing norm (Table 4). While the average thousand seed weight in the study was 6.85 g, the highest average thousand seed weight was 7.41 g from the Karakız cultivar, and the lowest was obtained from the Beyazgelin variety with 6.53 g (Table 4). According to the sowing norm, the thousand-grain weight varied between 6.33 g and 7.83 g, although it was not statistically significant. According to the sowing norm, the highest thousand seed weight average was observed from the 200 seeds/m² sowing norm of the Karakız variety, while the lowest thousand seed weight average was observed from the 1200 seeds/m² sowing norm of the Beyazgelin variety.

In our research, the thousand-grain weight varied between 6.53 and 7.41 among the varieties, and these values were 3.0-8.6 g according to Yıldırım (1998), 4.9-8.3 g according to Akçalı Can (1999), and Siddique et al. (2002) 4.6-4.7 g, according to Kurt et al. (2006) 1.6-6.3 g, according to Tunçtürk (2007) 5.3-6.2 g, and according to Endes (2010) 4.5-6.2 g. higher and more harmonious.

Crude oil rate

In the experiment, the changes in the crude oil ratio of linseed were found to be statistically significant at the level of 1%, depending on the sowing norm, but it was determined that it was not significant according to the interaction of cultivar and cultivar sowing norm (Table 4.25). The average crude oil rate in the study was 36.30%. Among the linseed varieties, the highest crude oil rate was 36.60% and the lowest crude oil rate was 35.92% from Beyazgelin variety (Table 4.26). According to the sowing norm, the average crude oil ratio changed between 34.90% and 37.39%, and the highest crude oil ratio was 37.39% from

the 200 seeds/m² sowing norm, while the lowest crude oil ratio was observed between 34.90% and 1200 seeds/m² sowing norm. Crude oil ratio values obtained in the study varied between 34.05% and 37.90%. These values obtained are 47.0-47.6% (Uzun 1992), 41.1-44.5% (Diri 1996), 40.7% (Qiang et al. 1996), 45.9% (Diri and Aslan 1997), 45-50% (Atakişi 1999), 41.1-47.0% (Akçalı Can 1999), 40% (Lukaszewicz et al. 2004), 30.7-46.6% (Gür 1998), 30.0-37.2% (Yıldırım 2005), 36.6-42.1% (Kurt et al. 2006), 28.9- 35.2% (Tunçtürk 2007), 31.2-38.7% (Yılmaz et al. 2007).

Seed yield

In the study, it was determined that the change in the seed yield (kg/da) of the linseed plant was statistically significant at the level of 1% according to the cultivars and sowing norm (Table 5). While the average seed yield was 118.06 kg/da in the study, the highest seed yield was 123.4 kg/da among the linseed cultivars and Beyazgelin cultivar had the lowest yield with 110.2 kg/da (Table 5). According to the sowing norm, the average yield ranged between 143.1 and 81.44 kg/da, and the highest seed yield was obtained from the sowing norm of 1000 seeds/m² and the lowest yield was obtained from the sowing norm of 200 seeds/m². The seed yield values obtained in the study varied between 67.46 kg/da and 147.97 kg/da between applications. Our seed yield results less than 140.8-235.4 kg/da (Akçalı Can 1999), 109.7-274.7 kg/da (Kurt et al. 2006) and 180 kg/da (Yılmaz et al. 2007) and higher than 31.1-57.9 kg/da (Ghatak et al. 1990), 45.9-52.1 kg/da (Geleta 1999) and 52.7-84.0 kg/da (Yıldırım 2005), at the same time similar with 80-195 kg/da (Gubbels and Kenaschuk 1989),(Khander and Sharma 1990), 81-127 kg/da (Yadav et al. 1990), 56.6-93.1 kg/da (Bassi and Badiyala 1992), 59.1-79.9 kg/da (Uzun 1992), 104-159 kg/da (Dubey and Singh 1994), 23.34-123.5 kg/da (Diri 1996), 72.9-142.5 kg/da (Qtang et al. 1996), 40-163 kg/da (Yıldırım 1998), 99.7-149.0 kg/da (Tunçtürk 2007) and 70-118 kg/da (Endes 2010).

Table 5. Variance analysis results and means of crude oil rate, seed yield, crude oil yield.

		Crude oil rate (%)	Seed yield (kg/da)	Crude oil yield (kg/da)
Replication	2	0.828öd	171.788öd	30.25öd
Cultivar (A)	2	2.167öd	867.898**	155.033**
Error1	4	1.88	10.764	4.302
Sowing rate (B)	5	6.725**	5938.967**	656.936**
AxB	10	0.536öd	77.678öd	9.055öd
Error	30	0.747	100.166	12.676
Cultivars				
Karakız		36.38	120.6A	43.73A
Beyazgelin		36.6	123.4A	45.01A
Sarıgelin		35.92	110.2B	39.40B
Sowing rates (seeds/m^2)				
200		37.39A	81.44C	30.48C
400		36.92AB	92.50C	34.17C
600		36.52AB	118.5B	43.27B
800		36.19AB	135.2A	48.88A
1000		35.92BC	143.1A	51.37A
1200		34.90C	137.8A	48.10A
Cultivars x Sowing rates (seeds/m^2)				
Karakız	200	37.19	87.19	32.44
	400	37.06	98.29	36.42
	600	37.03	118.22	43.82
	800	36.42	136.43	49.65
	1000	35.96	144.73	52
	1200	34.65	138.46	48.02
Beyazgelin	200	37.9	89.65	34
	400	37.17	101.02	37.51
	600	36.29	120.48	43.64
	800	36.07	143.1	51.6
	1000	36.19	147.97	53.56
	1200	35.97	138.26	49.69
Sarıgelin	200	37.06	67.46	24.98
	400	36.5	78.17	28.58
	600	36.24	116.76	42.35
	800	36.06	125.92	45.39
	1000	35.61	136.44	48.53
	1200	34.05	136.54	46.58

In the experiment, it was determined that the change in crude oil yield (kg/da) of linseed was statistically significant at the level of 1% according to the cultivars and sowing norm (Table 5). In the study, the average crude oil yield was 42.71 kg/da, and the highest crude oil yield was obtained from Beyazgelin cultivar with an average of 45.01 kg/da, while the lowest average

crude oil yield was in Sarıgelin variety with 39.40 kg/da (Table 5). According to the sowing norm, the average crude oil yield ranged between 30.48 and 51.37 kg/da, and the highest average oil yield was observed from the 1000 seeds/m² sowing norm, the lowest was 30.48 kg/da from 200 seeds/m² sowing norm. Crude oil yield varied between 24.98 and 53.56 kg/da according to the applications, and these values are lower than 78.0 kg/da Yilmaz et al., (2007), and similar with 35.16-55.03 kg/da (Yadav et al. 1990), 28.2-38.5 kg/da (Uzun 1992), 10.09-56.78 kg/da (Diri 1996), 15.83-29.93 kg/da (Yıldırım 2005) and 32.5-50.8 kg/da (Tunçtürk 2007).

CONCLUSION

All the research results were evaluated, the plant height of the Karakız cultivar was longer than the other cultivars and these differences were found to be statistically significant. The number of capsule and seeds in capsule were differed according to both variety and sowing norm, and a decrease in the number of capsule and seeds in capsule determined with the increase in sowing norm. In terms of cultivars, the highest number of capsules was obtained from Sarıgelin cultivar, while the highest number of seeds in the capsule was determined from Beyazgelin cultivar. In terms of capsule width, Karakız variety reached the highest (7.48 mm) sowing norm of 200 seeds/m2, while 1200 seeds/m² planting norm of Sarıgelin cultivar provided the lowest (6.96 mm) capsule width value.

While only the difference between the cultivars was statistically significant in the capsule length feature, the highest capsule length was observed in the Karakız variety with 8.67 mm. Thousand seed weight was effected from both cultivar and sowing norm and this changes was statistically significant. Karakız variety provided the highest thousand seed weight with 7.41 g and 200 pcs/m2 sowing norm with 7.24 g. While the difference between the varieties in terms of crude oil ratio was not statistically significant, the sowing norm was significant and the increase in sowing norm caused a decrease in the crude oil ratio. Seed yield and crude oil yield were statistically significant both among varieties and according to sowing norm. While Beyazgelin cultivar reached the highest value in both seed and crude oil yield, it was included in the same group as the difference between it and Karakız cultivar was not significant. While the sowing norm of 1000 seeds/m2 had the highest value in

both seed and crude oil yield in the sowing norm, it was included in the same group with the sowing norms of 1200 seeds/m2 and 800 seeds/m2 because the difference between them was not significant. As a result of this study conducted with oilseed linseed varieties, Beyazgelin and Karakız varieties stood out in terms of both seed and crude oil yield, while it was observed that 1000 pcs/m2 sowing norm had better results than other applications. Since the study was only one year, the observation values were discussed, and it is necessary to repeat the study in other years for conclusive results.

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CHAPTER 6

DETERMINATION OF THE EFFECTS OF BIOTIC AND ABIOTIC FACTORS ON DECREASER SPECIES DISTRIBUTION IN RANGELAND VEGETATIONS BY THE POISSON REGRESSION

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INTRODUCTION

Plant development is affected by environmental factors in addition to genetic structure. A wide range of changes from soil structure to topography and climate has a direct or indirect effect on plant growth. If the changes in vegetation due to biotic or abiotic factors take place slowly and lightly, plants can adapt to these changes over time. However, it is difficult for the plants to adapt to rapid and violent changes, and they may withdraw from the vegetation. For a better definition and management of rangeland ecosystems, it is important to understand the relationships between environmental factors and vegetation (Brinkmann et al., 2009). The effects of grazing management, which is probably among the most common land use, on plant communities substantially vary due to the irregular nature of grazing systems (Arévalo et al., 2011).

Vegetation, an important part of the ecosystem, is most affected by the change process in rangeland ecosystems. Rangeland vegetation is affected by faulty rangeland management practices and grazing depending on its severity (Deng et al., 2014). Intensive grazing reduces the total vegetation cover and significantly alters the botanical composition (Hanke et al., 2014). Grazing exerts a substantial effect on steppe yield and the grassland ecosystem (Ren et al., 2016). Grazing affects resident species composition and coverage ratios, however, soil characteristics and climate can have a more substantial effect on plant species diversity than current grazing levels (Stohlgren et al., 1999).

As well as biotic factors, abiotic factors such as slope, elevation, aspect, soil properties, and climate also possess key roles in shaping rangeland vegetation (Amezaga et al., 2004; Bennie et al., 2006). Species richness changes significantly as a result of the pressure from grazing in arid areas because climatic conditions and grazing are the main driving forces of botanical composition and species richness in arid rangeland ecosystems (Cheng et al., 2011). However, topographic effects cannot be overlooked when examining the effects of climate on vegetation (Li & Guo, 2014). Altitude and slope are important topographic variables. Altitude affects species composition but does not significantly affect the number of species (Marini et al., 2007). Slope direction plays a critical role in affecting the vegetation pattern in semi-arid areas (Yang et al., 2020).

Comprehension of the plant community's response to combinations of biotic and abiotic factors is of great importance for the prediction of the ecosystem's response to environmental changes (Wood et al., 2012). The interaction of adverse abiotic conditions and inappropriate grazing management practices can be cited as the cause of the deterioration in rangelands. The results reported on the size and location of degraded rangelands provide the basis for the development of management plans for future restoration and appropriate use of rangeland resources (Pasho et al., 2014).

Before making rangeland management plans, vegetation characteristics including the covered area of the rangeland and the ratios of decreaser, increaser, and invader species in its botanical composition should be determined. Since the data in such studies are obtained by enumeration and observed within certain periods, a significant part of these data shows a Poisson distribution. The Poisson distribution determines the probability of encountering a plant in each area. Measuring the distances observed between plant species and varieties in vegetation studies increases the chance of success (Karadavut, 2022; Sellers et al., 2012). The negative effects on the plants in the rangeland increase or decrease depending on the duration and the intensity of the factors that cause deterioration in rangeland vegetation. The present study was conducted in Tokat province rangelands where no grazing system is applied and, consequently, a heavy grazing has been established and aimed to determine the effects of factors such as altitude, slope, aspect, erosion, grazing intensity, and the distance from the rangeland to the settlement on the decreaser species in the rangelands.

MATERIAL AND METHOD

General characteristics of the research area

The research data were obtained from the rangeland parcels in different locations within the borders of Tokat Province and determined according to the purposive sampling method. The rangelands in the study area were divided into four groups according to their slopes: flat and nearly flat (0.0-2.0%), slightly sloping (2.1-12.0%), medium sloping (12.1-30.0%), and high sloping (30.1% and above). The vegetation studies were carried out at 101 stops. Of the stops where vegetation studies were conducted, the slopes of 23 stops were flat and nearly flat, the slopes of 58 stops were slightly sloping, and the slopes of 20

stops were moderately sloping. The number of stops at 0–750 m altitude was 21, between 750–1250 m 29, between 1250–2000 m 48, and the number of stops above 2000 m was 3. In the study area, 26 stops were in the west, 20 stops in the east, 18 stops in the south, and 37 stops were in the north.

Vegetation Examination

A modified wheel point method with a loop was adopted to identify the species that make up the botanical composition in the research area (Koç & Çakal, 2004). During the vegetation examinations, factors such as changes in the rangeland areas, and aspect were considered. Each examined point was accepted as a stop, and a vegetation examination was carried out by reading plants at 400 points in total on four lines in the east, west, north, and south directions at each stop. In the research, 40400 readings were made at 101 stops. The ratios of each plant species read in the total number of plants were calculated, and the ratios of the species in the botanical composition were determined.

As a result of the vegetation studies, 294 different species were identified in the rangeland areas of Tokat province. Of the species, 58 (19.7%) were grasses, 65 (22.1%) were legumes and 171 (58.2%) were species belonging to other families. It was determined that 38 (12.9%) of the detected species were decreaser, 27 (9.2%) were increaser and 229 (77.9%) were invader species. The ratio of the vegetation-covered area of the rangelands where the research was carried out varied between 43.25 and 99.75%, the average area covered with vegetation was 86.2% and the bare area ratio was calculated as 13.8%. In terms of rangeland conditions, 2.97% of the stops were excellent (3 stops), 12.87% were good (13 stops), 71.29% were fair (72 stops) and 12.87% were poor (13 stops) rangelands (Figure 1).

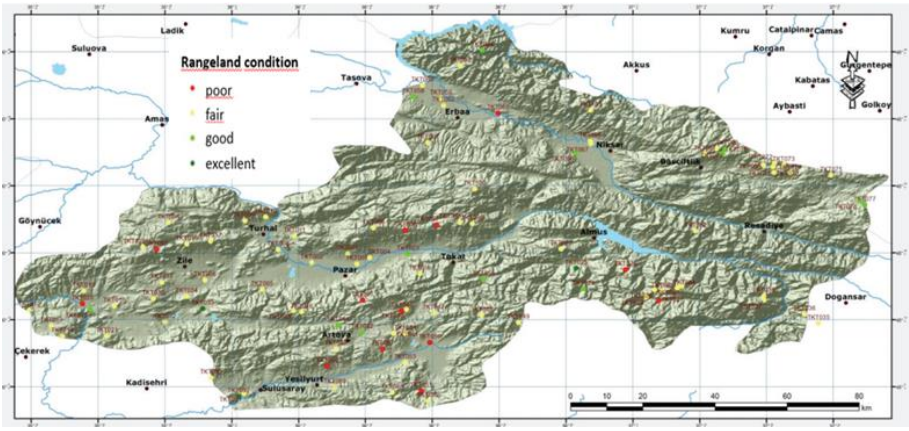

Figure 1. Rangeland conditions of the research area and distribution of the stops

In the study, of the 38 decreaser species identified in the rangelands, 32 species that were present in all the vegetation-examined stops were taken into the examination as the material. The names of these species and their abbreviations used in the figures are given in Table 1.

Table 1. Decreaser species examined in the study and their abbreviations

Species	Abbreviation	Species	Abbreviation
Agropyron intermedium	Agr int	*Medicago sativa*	Med sat
Alopecurus arundinaceus	Alo aru	*Medicago varia*	Med var
Alopecurus pratensis	Alo pra	*Onobrychis armena*	Ono arm
Arrhenatherum elatius	Arr ela	*Onobrychis galegifolia*	Ono gal
Bothriochloa ischaemum	Bot isc	*Phleum montanum*	Phl mon
Bromus erectus	Bro ere	*Phleum pratense*	Phl pra
Bromus inermis	Bro ine	*Poa longifolia*	Poa lon
Bromus tomentellus	Bro tom	*Poa nemoralis*	Poa nem
Bromus variegatus	Bro var	*Poa pratensis*	Poa pra
Dactylis glomerata	Dac glo	*Trifolium fragiferum*	Tri fra
Festuca arundinacea	Fes aru	*Trifolium hybridum*	Tri hyb
Festuca pratensis	Fes pra	*Trifolium montanum*	Tri mon
Koeleria cristata	Koe cri	*Trifolium pannonicum*	Tri pan
Lolium perenne	Lol per	*Trifolium pratense*	Tri pra
Medicago falcata	Med fal	*Trifolium repens*	Tri rep
Medicago papillosa	Med pap	*Trifolium trichocephalum*	Tri tri

Statistical Analysis

The data obtained as a result of vegetation studies were evaluated by adopting the Poisson regression analysis. The results of the Poisson regression analysis were subjected to G^2 and R^2 goodness-of-fit tests to test the success of the established model. The effect levels of the factors affecting the species distribution were determined by considering the Euclidean distances obtained by using the Poisson distribution (Deniz, 2005; Dobson, 2018; Ozmen, 2000).

The Poisson regression model used in the research was as follows.

$$\frac{c}{N} = e^{\alpha+\beta_i x_i+\varepsilon_i}$$

c: The number of plant species under the effect of geographical location,

N: The total number of plant species,

α : Fixed term

β_i : The regression coefficient of the explanatory variable,

x_i : i. descriptive variable,

ε : Error term,

e : 2.1718 (Base of natural logarithms)

The G^2 statistic used to measure the goodness of fit was given below.

$$G^2 = 2\sum_{i=1}^{n} y_i \ln\left(\frac{y_i}{\mu_i}\right)$$

The measure equation for the Poisson regression model R_p^2 was presented below.

$$R_p^2 = 1 - \frac{\log L(y) - \log L(\hat{\mu})}{\log L(y) - \log L(\overline{\mu})}$$

In this research, the effects of biotic (grazing intensity) and abiotic (altitude, slope, erosion, aspect, and distance to settlement) factors on the distribution of decreaser species in rangelands were determined.

RESULTS and DISCUSSION

Inter-factorial Relationships

It was revealed that there was a negative relationship between altitude and erosion and grazing intensity, whereas a positive relationship between aspect and erosion and grazing intensity (Table 2). There was a positive relationship between slope and erosion, between erosion and grazing intensity, and distance from the settlement. Furthermore, there was a negative correlation between the slope and the grazing intensity, and the grazing intensity and the distance to the settlement (Table 2).

Table 2. Relationships between the factors affecting the ratio of decreaser species

	Altitude	Aspect	Slope	Erosion	Grazing Intensity	Distance to Settlement
Altitude	1.000	0.112	0.316	-0.616**	-0.542**	0.166
Aspect		1.000	-0.21	0.562**	0.766**	0.217
Slope			1.000	0.795**	-0.558**	0.313
Erosion				1.000	0.769**	0.618**
Grazing Intensity					1.000	-0.458*
Distance to Settlement						1.000

*p<0.05, ** p<0.01

It was also revealed that erosion and grazing intensity decreased as the altitude increased. Sabahaddin et al. (2011) have reported that there was a close relationship between grazing density and erosion, and as the altitude increased, the grazing density and the effect of erosion decreased. Erosion and grazing intensity increased as the aspect changed (Table 2). This result was associated with the changes in the direction of grazing from north to south or from south to north. Because the ecological conditions and, therefore, the vegetation structures of the rangelands facing north and south were different from each other. Yang et al. (2020) emphasized that the effect of direction on vegetation characteristics was significant and that the north-facing slopes were superior to the south-facing slopes in terms of vegetation, biomass, and species diversity.

As the slope increased, the erosion increased significantly, whereas the increase in the slope led to a decrease in the grazing intensity. Sun et al. (2014) have reported that, as the slope increases, soil erosion increases significantly. In addition to the increase in the slope, the grazing density decreased and the structure of the vegetation changed with the effect of the north direction (Amezaga et al., 2004). The grazing intensity decreased as the distance of the rangeland to the settlement increased, while the erosion increased when the grazing intensity increased, and the grazing rangeland was closer to the settlement. (Table 2). Pasho et al. (2014) have stated that erosion is related to the grazing intensity and distance to the village, the degradation of rangeland characterized by bare soil was higher in rangeland s close to village settlements and roads.

Altitude

It was determined that the ratios of decreaser plant species in the botanical composition varied depending on the altitude and the responses of species to changes in altitude were very different (Figure 2). The species *Trifolium repens, Trifolium hybridum, Trifolium pratense, Bromus inermis, Poa pratensis, Bromus variegatus*, and *Poa nemoralis* were positively affected by the altitude, and their ratios in the botanical composition increased as the altitude increased. The ratios of *Trifolium pannonicum Trifolium montanum Trifolium trichocephalum, Medicago varia, Lolium perenne*, and *Phleum pratense* in the botanical composition decreased with the increase in altitude. The species *Medicago sativa, Onobrychis galegifolia, Medicago falcata, Medicago papillosa, Koeleria cristata, Festuca arundinacea*, and *Phleum montanum* were in general affected by the altitude, however, it can be argued that the effect of altitude on these species was not significant (Figure 2).

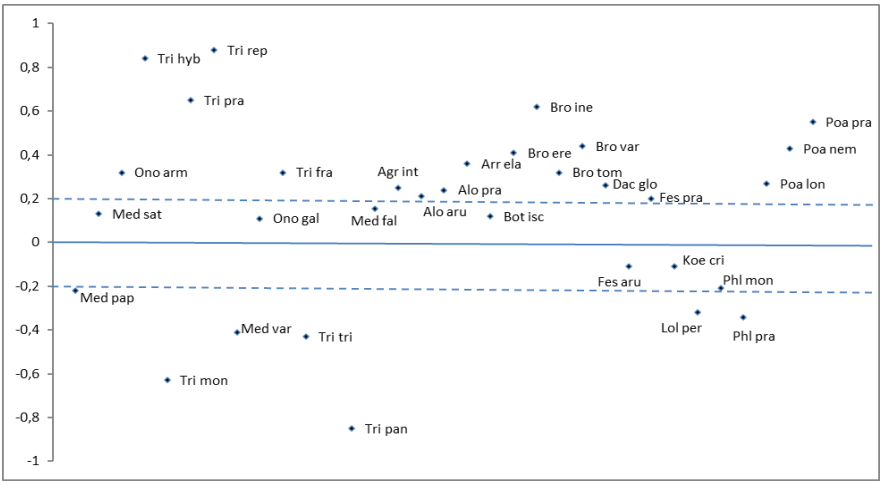

Figure 2. The effect of altitude on the ratios of the decreaser species.

The increase in altitude caused a decrease in grazing intensity and an increase was observed in the ratio of a significant part of the species (Table 2, Figure 2). The main environmental factors affecting the changes in botanical composition are altitude and soil depth (Vassilev et al., 2011). The ratios of legumes and grasses in the botanical composition are higher in low-altitude rangelands than those in high-altitude rangelands (Oztas et al., 2003). The positive relationship between plant species richness and biomass is most affected by altitude (Bhandari & Zhang, 2019). Although altitude and slope affected the species composition, it did not change the number of species (Marini et al., 2007).

Slope

An increase or a decrease in the slope had no effect on the ratio of *Medicago papillosa*, *Trifolium repens*, *Medicago falcata*, *Onobrychis galegifolia*, *Trifolium pratense*, *Bromus erectus* and *Koeleria cristata* in vegetation (Figure 3).

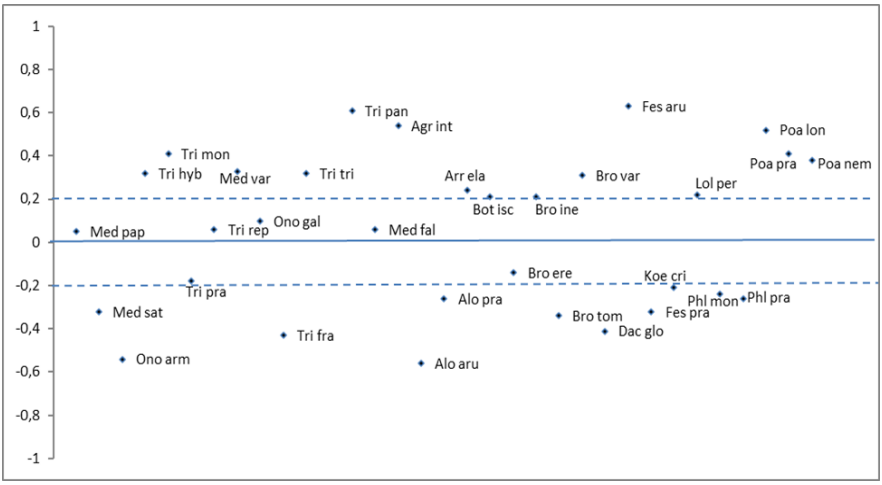

Figure 3. The effect of the slope on the ratios of the decreaser species.

The ratios of *Festuca arundinacea, Trifolium pannonicum, Agropyron intermedium, Poa longifolia, Trifolium montanum, Poa pratensis*, and *Poa nemoralis* increased in the botanical composition as the slope increased. The ratios of *Alopecurus arundinaceus, Onobrychis armena, Trifolium fragiferum*, and *Dactylis glomerata* in the botanical composition decreased as the slope increased (Figure 3).

A topographic factor, the slope is highly associated with the species richness (Li & Guo, 2014; Marini et al., 2007)and changes in slope affect species composition (Gong et al., 2008). Unfavorable area characteristics including poor soil quality and steep slopes are important factors that affect species diversity (Klimek et al., 2007). The effect of the slope on yield and quality is caused by the soil layer on the slopes, which is eroded by rainfalls. As a result of erosion, more productive vegetation that can stay green for a longer period may develop due to the soil accumulating in the lower sections, and the vegetation on the slopes may be less productive (Sürmen & Kara, 2018).

Erosion

The increase in erosion intensity instigated a significant decrease in the ratios of *Medicago papillosa, Medicago sativa, Trifolium montanum, Medicago falcata, Trifolium fragiferum, Trifolium repens* and *Trifolium trichocephalum* in the botanical composition. There was a slight increase in the ratios of *Trifolium pannonicum* and *Festuca pratensis*. However, the increase in the

ratios of *Bothriochloa ischaemum* and *Festuca arundinacea* was not significant (Figure 4). On the other hand, the decrease in *Trifolium pratense, Alopecurus arundinaceus, Bromus inermis, Bromus tomentellus*, and *Poa longifolia* was also not significant. An increase or decrease in erosion did not cause any changes in the ratios of *Agropyron intermedium, Lolium perenne, Bromus variegatus, Arrhenatherum elatius, Phleum montanum, Phleum pratense, Alopecurus pratensis, Bromus erectus, Dactylis glomerata, Koeleria cristata, Poa pratensis, Poa nemoralis, Onobrychis armena, Onobrychis galegifolia, Medicago varia, and Trifolium hybridum* (Figure 4).

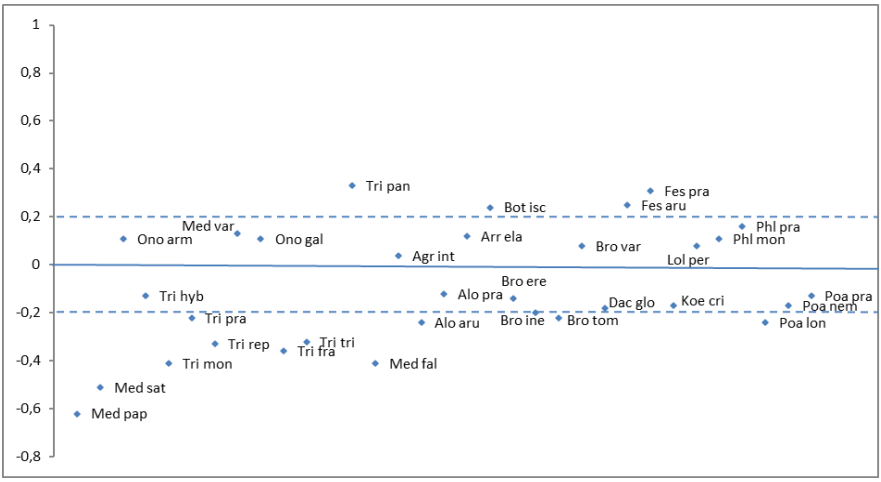

Figure 4. The effect of erosion on the ratios of the decreaser species.

The results obtained in the present study revealed that the distribution of species changes depending on the severity of erosion and that legumes were more adversely affected than grasses. Because the grasses except for *Festuca pratensis*, *Festuca arundinacea*, and *Poa longifolia* were not significantly affected by the changes in erosion (Figure 4). Stem and massive fibrous roots of grasses are more effective in both preventing erosion and resisting erosion conditions (Maiti & Maiti, 2014). Severe erosion, along with slope and nutrient deficiency, causes a decrease in species richness, changes in composition, and deterioration of vegetation (Jiao et al., 2009; Pasho et al., 2014). However, the intensity of erosion is significantly affected by the changes in vegetation (Chen et al., 2018).

Aspect

The changes in the aspect from south to north significantly increased the ratio of *Onobrychis armena* in the botanical composition. Furthermore, *Festuca arundinacea*, *Alopecurus pratensis*, and *Festuca pratensis* were also positively affected by the changes in the aspect (Figure 5). The changes in the aspect from north to south did not affect the ratios of grasses in the botanical composition, however, it generally negatively affected the species belonging to the legume family.

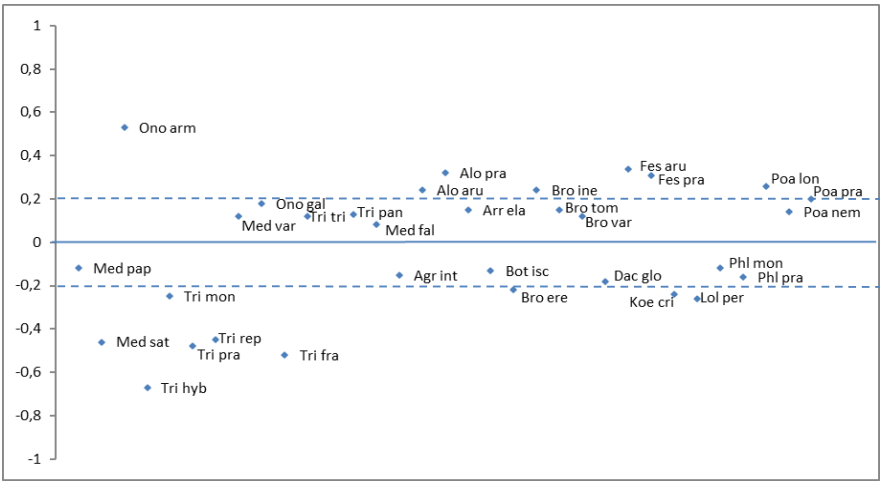

Figure 5. The effect of aspect on the ratios of the decreaser species.

The ratios of *Trifolium hybridum*, *Trifolium pratense*, *Trifolium repens*, *Trifolium fragiferum*, and *Medicago sativa* in the botanical composition decreased significantly as the aspect changed (Figure 5). Erosion and grazing intensity were significantly adversely affected by the changes in the aspect from north to south (Table 2). Also, the effect of the vegetation on the species was substantially varying and the most adversely affected species from the changes in the aspect were the legumes (Figure 5).

The northern slopes have higher yields and species diversity than the southern slopes, due to the strong impact of the aspect on the yield and species composition of the rangeland (Gong et al., 2008; Yang et al., 2020). The resistance of vegetation to the vegetational changes on south-facing slopes was weaker than that on the northern slopes (Bennie et al., 2006). Aspect is one of the important factors affecting the species richness and the vegetation-covered

area (Gelbard & Harrison, 2003). It is an important determinant of species distribution in the slope and vertical vegetation, which can vary within short distances (Bennie et al., 2008).

Grazing Intensity

As the severity of grazing increased in the study area, the ratios of legumes *Trifolium hybridum, Medicago sativa, Trifolium pratense, Medicago varia*, and *Trifolium repens* in the botanical composition significantly decreased (Figure 6). The increase in grazing intensity also instigated a significant decrease in the ratios of *Festuca pratensis, Dactylis glomerata, Agropyron intermedium, Phleum pratense*, and *Festuca arundinacea.* A lower decrease was observed in the ratios of the species *Onobrychis galegifolia, Medicago falcata, Medicago papillosa, Trifolium montanum, Trifolium trichocephalum, Bromus inermis, Bromus variegatus*, and *Koeleria cristata* compared to other species that were significantly affected by the increase in grazing intensity. However, the ratio of species such as *Bromus tomentellus, Bromus erectus, Alopecurus arundinaceus, Poa nemoralis, Alopecurus pratensis, Onobrychis armena* and *Trifolium fragiferum* in the botanical composition was not affected by the changes in grazing intensity (Figure 6).

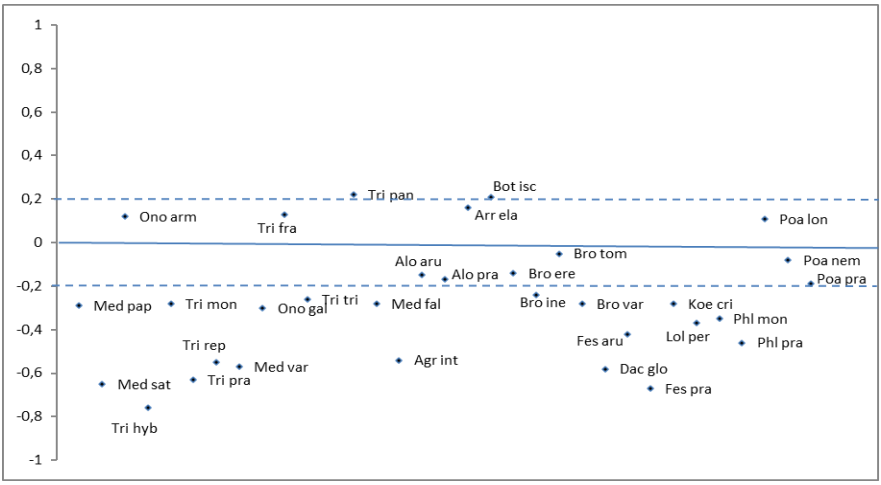

Figure 6. The effect of grazing intensity on the ratios of the decreaser species.

Legume and grass family species were similarly affected by the increase in grazing intensity. This negative effect was particularly concentrated in legumes. The results showed that there were no species that were positively affected by the increase in grazing intensity. (Figure 6). The similar negative effects of the grazing intensity on the examined species were associated with the similar tastes of the species in question or their equal preference by herbivores. Under overgrazing conditions, the selective grazing behavior of herbivores decreases (Murray, 1997). The palatability off species is an important factor affecting the species' composition and degradation in rangelands (Wu et al., 2008; Yan & Lu, 2015). Decreaser species are favored primarily by herbivores, while increases graze less frequently and invaders do rarely. The contribution of decreaser species to the botanical composition decreases due to increasing grazing intensity. First, the increaser and then the invader species become dominant in the vegetation (Koç et al., 2021). The dominance of invader species in the vegetation causes irreversible changes in the vegetation by affecting the abundance of other species (Ar et al., 2022). The decrease in the grazing density in rangelands increases species richness (Deng et al., 2014; Peco et al., 2006; Škornik et al., 2010). Because grazing density is solely the most important factor affecting diversity (Reitalu et al., 2010).

The distance from the rangeland to the settlement

The changes in the distance from the rangeland where herbivores graze from the settlement had no effect on the ratios of *Trifolium fragiferum, Agropyron intermedium, Alopecurus arundinaceus, Alopecurus pratensis, Arrhenatherum elatius, Lolium perenne, Phleum pratense, Phleum montanum* and *Festuca arundinacea* (Figure 7). On the other hand, the ratios of 23 species other than these species in the botanical composition increased as the distance from the rangelands to the settlements increased. This increase was particularly evident in the decreaser species in the legume family. The increase in the distance from the grazing rangelands to the settlements significantly increased the ratio of *Trifolium pratense, Medicago sativa, Trifolium repens, Trifolium trichocephalum, Medicago falcata, Trifolium montanum, Trifolium hybridum, Onobrychis galegifolia* and *Medicago papillos*a in the botanical composition. Also, P*oa longifolia, Poa nemoralis, Bromus variegatus, Bromus erectus,*

Bromus tomentellus, *Bromus inermis*, and *Poa pratensis* are the grass species with the highest increase in the ratio of botanical composition (Figure 7).

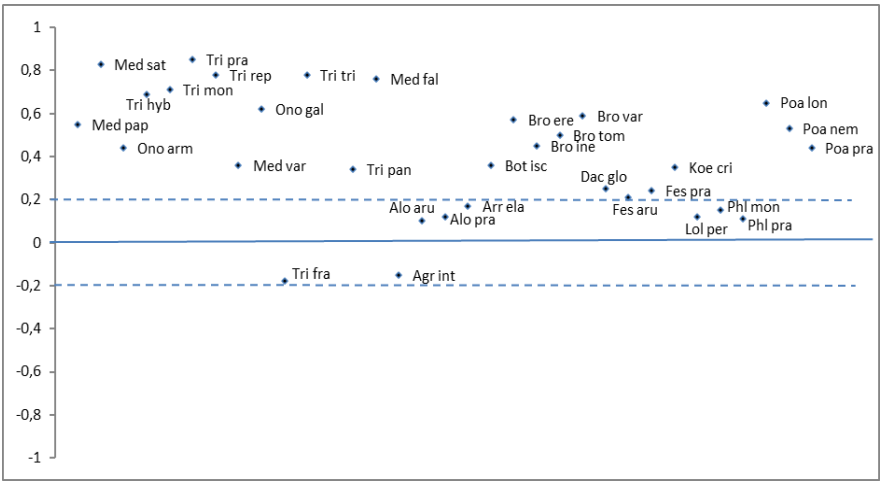

Figure 7. The effect of the distance from the rangeland to the settlement on the ratios of the decreaser species.

Since the grazing intensity decreases as the distance to the settlement increases, the ratios of the species that were negatively affected by the grazing intensity in the botanical composition increased. In other words, the examined species were generally positively affected by the increase in the distance of the rangelands to the settlement (Figure 7). This result was associated with the decrease in the grazing intensity as a result of the increase in the distance. Because there was a negative correlation between the grazing intensity and the distance of the rangeland to the settlement (Table 2). Following the grazing density, the distance to the rangeland has the greatest effect on species richness (Reitalu et al., 2010). As the distance to the rangeland increases, a significant increase occurs in the covered area and the number of species (Gelbard & Harrison, 2003). Grazing affects plant type, life form, distribution, and cover, but the distance from rangeland is very important (Stohlgren et al., 1999).

CONCLUSION

Understanding the plant community's response to combinations of biotic and abiotic factors is of great importance for the prediction of the ecosystem's response to environmental changes (Wood et al., 2012). The results showed that the factors such as altitude, slope, erosion, aspect, grazing intensity, and the

distance to the settlement have a significant effect on the distribution of the decreaser species in the rangeland vegetation. The effects of each of these factors, solely or together, were various. Evaluating each factor solely, those that most negatively affect the decreaser species rates were grazing intensity, erosion, and aspect, in order of importance. The increase in the slope increased the erosion whereas decreasing the grazing intensity. The increase in the altitude and the distance to the settlement positively affected the decreaser species distribution. The changes in aspect from south to north positively affected the ratios of decreaser species, whereas the changes from north to south had a negative effect. The results regarding the effects of the biotic and abiotic factors that were examined in the study on the ratios of the decreaser species will be a source for the conservation of vegetation, the planning of grazing systems, rangeland improvement, and sustainable rangeland management.

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CHAPTER 7

ITS (Internal Transcribe Spacer) REGION: A RELIABLE BARCODE FOR PHYLOGENETIC RECONSTRUCTIONS IN PLANTS

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INTRODUCTION

In humans' life, classification is an important step. All things can be classified based on some different characters even if they are morphological or not. In living organisms, since biology were accepted as a science, all have been classified. For conserving and using plants, description is the first step for scientists (China Plant BOL Group 2011). Botanists have used many characters especially morphological characters to classify plant species but in recent days chromosomes, proteins and DNA sequences are begun to use (Judd et al. 2008). Nonetheless, identification of plant species still very complicated. Especially within closely related species which have hybridization, intraspecific variation, radiation, can associate these description problems because of huge species divergence in plants (Stebbins 1950; Rieseberg et al 2006). In the same family members, there are mostly concerted evolution that indicate higher similarity to each other to orthologous genes in related species. Therefore, DNA barcoding approach becomes a popular method for describing species based on short, standardized DNA fragments which shows a unique path for identifying plants (Hebert et al 2003; Hebert and Gregory 2005).

In recent years, after invention of DNA sequences, molecular identification of plants in systematics became a valuable issue for studying. Not only total genomic DNA, also plastid and mitochondrial DNAs are often used for identification of species. In plants especially, due to its low substitution rates, mitochondrial DNA is rarely used. However, many regions of plastid DNA have been studied for universality and discrimination power in barcoding plants due to the majority of the plastid genome (Kress et al 2005; Kress and Erickson 2007; Fazekas et al 2008; Hollingsworth et al. 2009).

An ideal DNA barcode has got some important characters like universal, cost-effective, reliable and indicate well discrimination (CBOL Plant Working Group 2009). The CBOL Plant Working Group advised the international plant barcoding community to try to further evaluate plant barcodes and basically to standardize a DNA barcode for plants (Hollingsworth 2011). For plastid DNA markers, none of the single marker can be satisfy all needs for plant systematics. Therefore, combination of plastid markers should be solving the problems i.e., rbcL + matK or rbcL + matK + trnH–psbA (Fazekas AJ, et al. 2008; CBOL Plant Working Group 2009). In August 2009, the 2-marker combination rbcL + matK was suggested as the core barcode for land plants (CBOL Plant

Working Group 2009). Nonetheless this combination was the result of the study on few number species that have multiple samples where were colected from multiple congeneric species. Moreover, in November 2009 at the 3rd International Barcoding of Life Conference in Mexico City it was reported that combination of core barcodes (rbcL and matK) should evaluated from both the plastid genome (e.g., trnH–psbA) and the nuclear genome (e.g., ribosomal DNA ITS1 or ITS2) (China Plant BOL Group 2011).

INTERNAL TRANSCRIBE SPACER (ITS) REGIONS

Concerted evolution is conspicuous in multicopy nuclear genes in which homogenization is accomplished during meiosis by unequal crossing over (Dover 1994; Ganley and Kobayashi 2007; Osuna-Mascaró et al. 2022). The 45S nr ribosomal DNA is the best defined multicopy gene families with its hundreds to thousands of copies of tandem repeats in only single loci at each gene (Osuna-Mascaró et al. 2022). Moreover, rDNA which has different parts used in biodiversity studies, is completely very useful in phylogenetic studies (Hamby and Zimmer 1992). In conservation studies, 3 different subunits of the r-gene that has useful size for phylogenetic studies are examined at different levels of taxonomy (Appels and Dvorak 1982; Saghai-Maroof et al. 1984; Yu Sun et al 1994). The 18S, ITS1, 5.8S, ITS2 and 26S rDNA which are divided with non-transcribe IGS (intergenic spacers) form the whole region on rDNA (Fig 1) are the main sub regions (Sone et al. 1999). The ITS1 and ITS2 which named as internal transcribe spacers are the most advantageous part within all these regions in phylogenetic studies with their such characteristics like biparental inheritance, more evolutionary rates than other regions and the smaller in length (Long and Dawid 1980; Baldwin et al. 1995; Álvarez and Wendel 2003; Ganley and Kobayashi 2007). Furthermore, even if ITS sequences display fast concerted evolution at low levels of intra-genomic nucleotide divergence at short polymorphic events, nucleotide homogenization persists beyond ITS sequences more in plants than animals. Therefore, this situation results in almost high intra-genomic polymorphism (Buckler and Holtsford 1996; Mayol and Rosselló 2001; Popp and Oxelman 2004; Harpke and Peterson 2006; Nieto-Feliner and Rosselló 2007; Denk and Grimm 2010; Xiao et al. 2010; Xu et al.2017; Osuna-Mascaró et al. 2022).

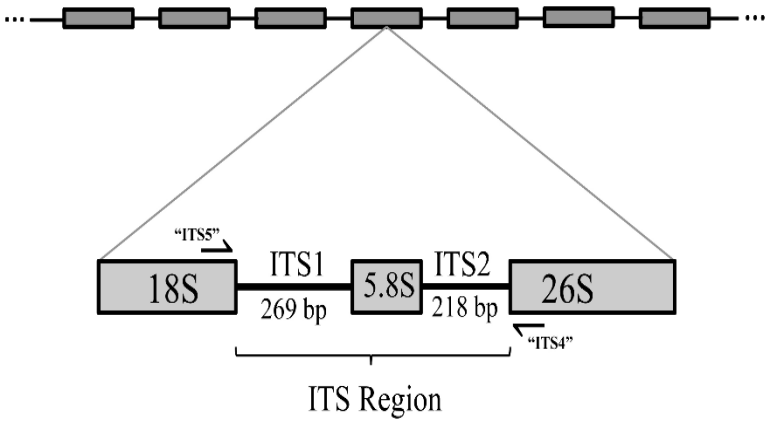

Figure 1: Structure of the ITS region of the nuclear ribosomal RNA genes (Zhang et al. 2015)

Between coding regions of 18S rRNA and 5.8S rRNA there is a gene region called ITS 1 in eukaryotes. ITS 1 is described as an ideal marker especially for plants at each level of taxonomic categories due to its comparatively high evolutionary rate at noncoding part. Moreover, the ease of amplification with universal primers at different organisms while studying the ITS1, gives an advantageous character to the region (Cherry et al., 1997; Ferris et al., 1994; Joyce et al., 1994; Nasmith et al., 1996; Nguyen et al., 2001; Szalanski et al., 1997, 2000; Wendt et al., 1993). ITS1 sequence gives sufficient and useful data for constructing phylogenetic analysis in many studies but sometimes resolving power within species of same genus alone is not reliable (Ye et al 2004).

The 5.8S which is a coding region between ITS1 and ITS2 region is the part of large subunit rRNA. Generally, the length of the region is varied between 163 to 165 bp in length (Hershkovitz and Lewis, 1996). This coding gene region also used as a phylogenetic marker at different taxonomic level with its motifs.

Another sub region is ITS2. Rather than other sub regions ITS1 and 5.8S, ITS2 is very important for plants due to its length, polymorphism rate, ease of

amplification, and cost effectiveness. In some plant species the beginning of the region is lack of genetic data because of contaminations with fungi, therefore, studying with ITS2 region mostly overcome this problem. So, even if it is a small region (approximately 200-220 bp in length), the region is sufficient for identifying high- and low-level taxa in plants (Petit and Excoffier 2009; Chen et al. 2010; Yao et al., 2010; Naciri et al., 2012; Han et al., 2013; Braukmann et al., 2017).

Not only using these regions primary sequences but also secondary structure forms are often used in phylogenetic analysis in plants. Especially ITS2 has high-order secondary structure which has a valuable implication in phylogenetic reconstructions. The ability of the structural constrains indicating the ITS2 is significant due to its implements on alignments and the waiting's used in phylogenetic data. After sequencing of ITS2 region, some modelling programs (etc. ITS2 server, mFold) are used to expect for secondary structure forms. At these predictions especially position of the hairpins is varied in distal positions within interbreeding organism. Therefore, these visual views of the sequences give scientists a clear evaluation about phylogenetic data of ITS2 addition to phylogenetic trees. So, it improves the phylogenetic utility of ITS2 region like morphological character in plant taxonomy (Hillis and Dixon 1991; Dixon and Hillis 1993; Sümer Ercan et al. 2022).

The combination of ITS regions (ITS1+5.8S+ITS2) have often been used in phylogenetic reconstruction studies in recent years. For instance, Karaman Erkul et al (2022) discovered a new species in *Astragalus* genus. They reported this new species by using both morphological data and phylogenetical data based on ITS regions. In that study, ITS regions were totally obtained as 611 bp in length and these nucleotides were enough for separating the species of different section of *Astragalus* genus. In the phylogenetic tree, which was drawn via ITS regions, new species separated from other species of the sections clearly (Fig2). As a conclusion it can be said that ITS regions can be suggested as a DNA barcode for new species discriminations that supported by morphological data.

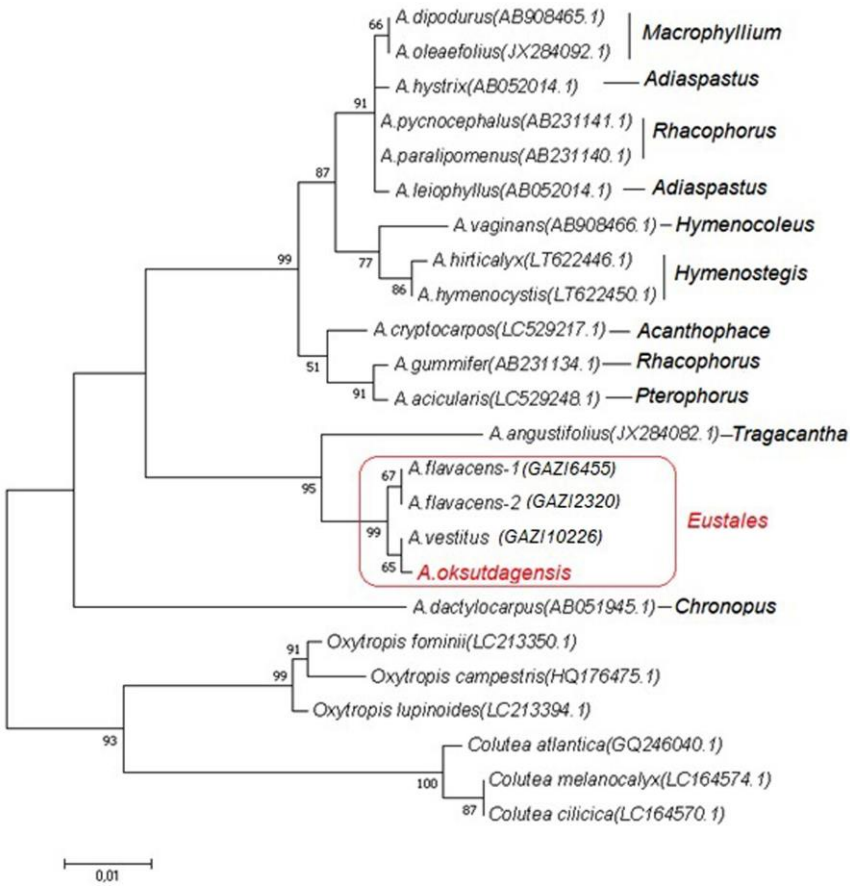

Figure 2: Phylogenetic tree of Astragalus species belongs to different sections based on ITS regions (Karaman Erkul et al. 2022).

Another example can be given on using ITS regions as a barcode for phylogenetic revisions at *Trifolium* genus (Sayed Ahmed et al. 2021). Sayed Ahmed et al. reported that using nr DNA ITS region and morphological data together gave a taxonomic perspective about the relationships among *Trifolium* species with reference to chromosomal divergences. ITS regions gave informative indels in combination with morphological data (Fig3).

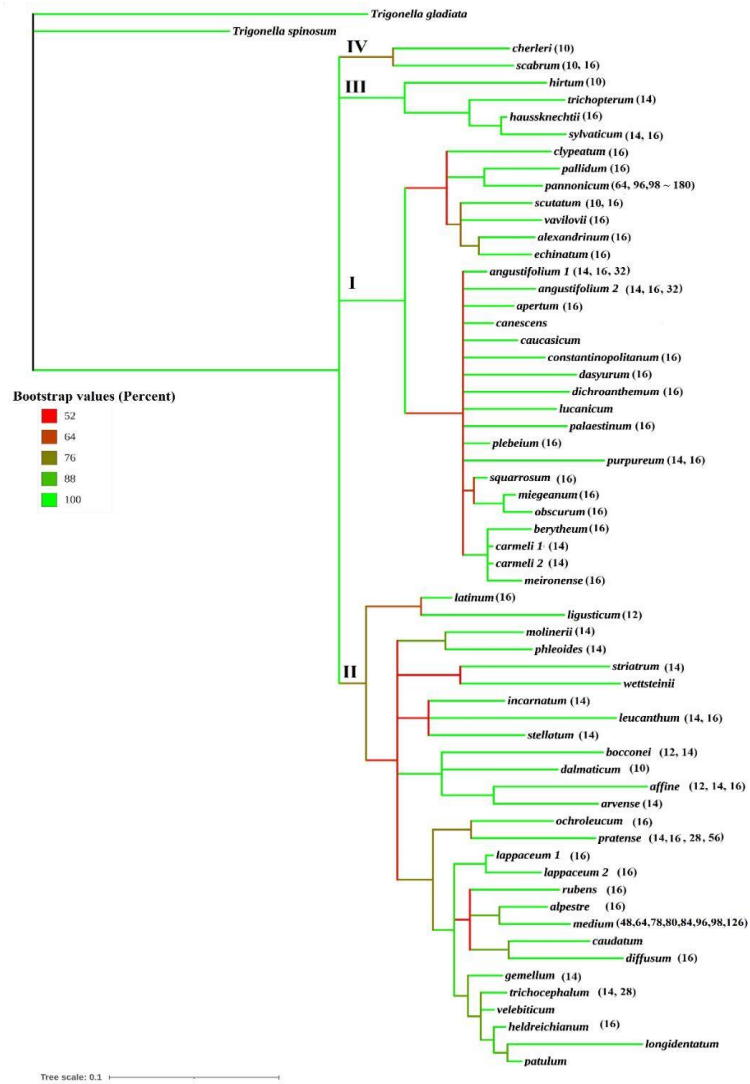

Figure 3 : ITS sequence tree that explained the molecular relationships of Trifolium genus (Sayed Ahmed et al. 2021).

Another study was done by Al-Juhani and Abdel Khalik (2021). Species of *Plectranthus* genus from Lamiaceae family were used as samples. They focused on 6 different *Plectranthus* species in which were endemic for Saudi Arabia. The 5 different plant barcodes were applied on the samples and one the barcode is ITS region from nrDNA. The results were evaluated separately and especially inter- and intraspecific distance, barcode gaps and Maximum

Likelihood analysis results were very satisfactory at ITS2 region even if it is shorter than other barcode regions. In the phylogenetic tree which was drawn by using only ITS2 region (Fig 4), indicated a well species divergence among *Plectranthus* species. Therefore, it could be safely said that, ITS2 region is a reliable barcode for plant phylogenetic studies due to its simplicity of amplifications.

One of the barcoding studies on Bamboo trees based on ITS regions were examined by Khanday et al. (2022). 18 bamboo species were studied, and phylogenetic results were obtained from ITS sequences. Even if morphological characters were indicated the divergence among Bamboo species, a clear perspective of all possible results of the study suggested that the ITS region sequence anlysis were used to combine with morphological ones. Moreover, molecular data helped to give us a view on taxonomical problems which were always talked about the renement of morphological character analysis (Fig 5).

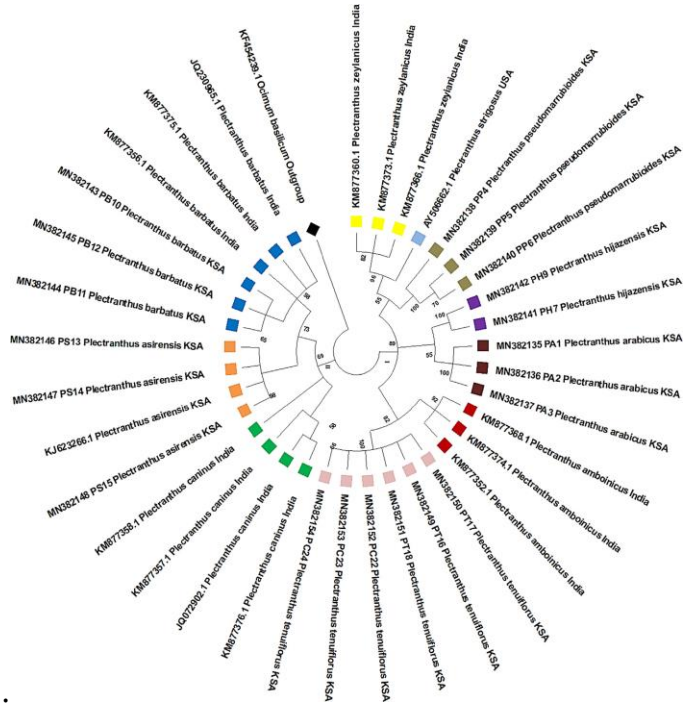

Figure 4: ITS2 sequence tree which indicated the relationships among Plectranthus spp (Al-Juhani & Abdel Khalik, 2021).

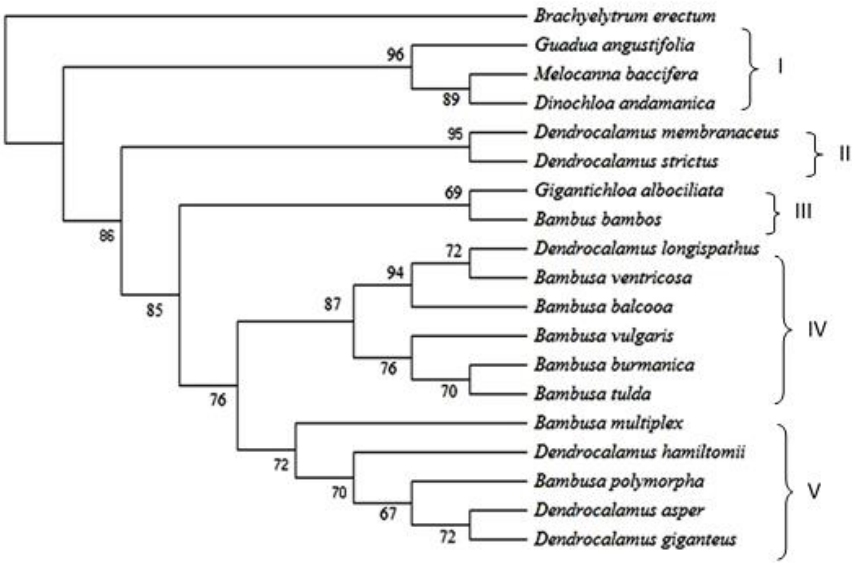

Figure 5: Phylogenetic reconstructions which were done by using ITS regions (Khanday et al. ,2022)

Addition to barcoding studies sometimes ITS regions were used for estimations of molecular divergence times in plants. Ateş et al. (2021) were focused on molecular time estimations at *Gundelia* sp which were naturally found in Turkey. The divergence time of the *Gundelia* sp were started in early Miocene (21.43 MYA: million years ago) and the most diverging species were separated at Pleistocene period ~1.8 MYA (Fig 6). Even if speciation still continues, the ancestral history of the genus was explained by using ITS regions.

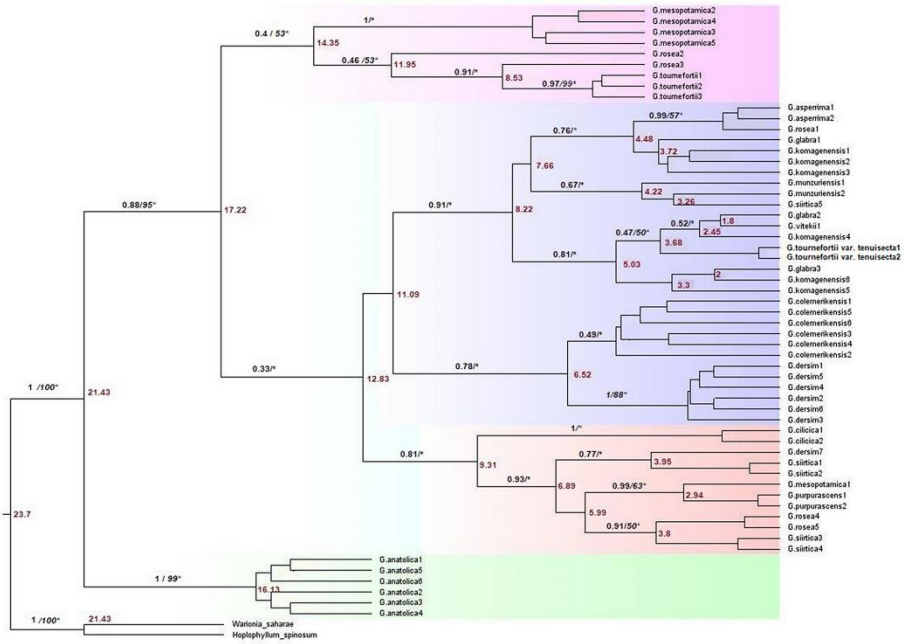

Figure 6: Molecular clock estimations of *Gundelia* sp. Based on ITS regions. Red numbers showed the divergence times (Ateş et al. 2021).

About secondary structure forms of ITS2 region, Sümer Ercan et al (2022) studied on phylogenetic descriptions of *Trichogramma* species in Turkey. With modeling program mFold web server, secondary structure forms of both *T. turkestanica* and *T. brassicae* were predicted (Fig 7). The genetic divergence among species in phylogenetic tree also reflected to secondary structure forms with hairpins, helices, and angles. Moreover, by using these angles the programs also analyze the ΔG (Gibbs) free energy values by using the secondary structure with thermodynamic calculations (Santa Lucia 1998) (Fig 7). As a result, it should be reported that secondary structure form of ITS2 region wshould be used like a morphological character because of its clear predictions of nucleotide diversities among species.

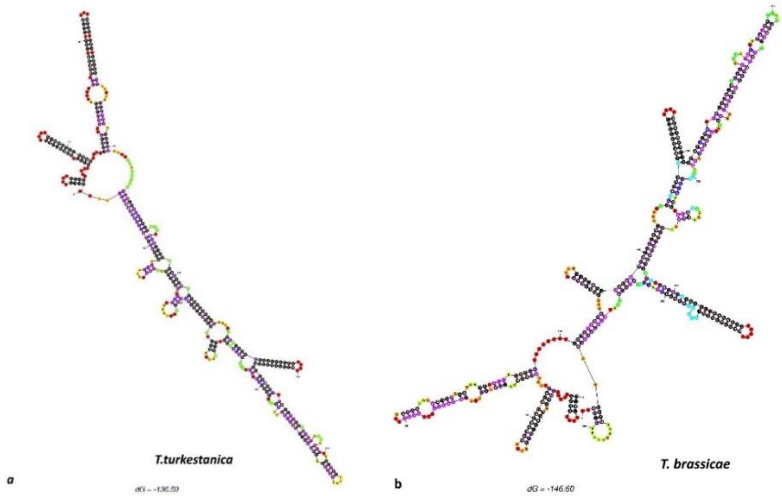

Figure 7: The estimated predictions of species based on 2ndary structure of ITS2 regions (Sümer Ercan et al. 2022).

CONCLUSION

After invention of DNA, using molecular data in every part of the science become a popular study. Especially after scanning of whole genomes even if they are genomic or plastid or mitochondrial, nucleotide sequences give us an evolutionary data about the history of the organisms.

Particularly, plant DNA regions whether they are coding or non-coding, presents a valuable view to the botanists that supports their morphological perspective. Therefore, using morphological and molecular data together become popular in recent studies due to the reliability of their combinations.

DNA barcoding markers which are used in plant phylogenetic studies are valuable for reconstructions. The claims on ITS regions as a universal barcode in plants, is very useful due to their well-documented in general terms. Whether it is a Angiosperm or Gymnosperm ITS region gives us a significant data on biodiversity. Description success at all levels gives the region as a reliable barcode (Chase MW, et al. 2007; Lahaye R, et al. 2008; China Plant BOL Group 2011). Ease of amplification, number of copies, universality of primer sets gives ITS a valuable implication. Especially using only one part of the region like ITS2 in problematic samples, also gives clear vision to

phylogenesis's. It seems that, using ITS as a barcode at any taxonomic level, can be useful to obtain reliable data even if they are contaminated or lost in time. In that cases discrimination power of ITS2 is seemed higher than other plastid markers even if they are lower than ITS per se due to its secondary structure forms.

As a conclusion, in plant systematic world in recent days, using DNA barcodes should be useful. In time the morphological characters have been exposed to many affects mostly mutationally by epigenetics. Therefore, using only morphological data is not gave clear vision, so, both morphological and molecular data are mostly useful for phylogenetic reconstructions. Moreover, using ITS region which have different sub regions is the mostly used DNA barcode in phylogenetic studies due to its valuable features. However, not only ITS regions also combination of the barcodes both plastids and genomics like ITS+mat K or ITS+ rbcL should be more useful in the phylogenetic perspectives on biodiversity studies.

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CHAPTER 8

PROPAGATION TECHNIQUES IN RHODODENDRONS

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INTRODUCTION

Rhododendron is a species of evergreen or deciduous shrub and rarely tree-shaped plants in the *Rhododendron* genus in the *Ericaceae* family (Küçük, 2005). In some deciduous rhododendron species, the green leaves turn from green to yellow and then to brown and red, displaying a beautiful autumn color appearance. *Rhododendron* flowers occur singly or in clusters at the ends of the shoots. These flowers are very large and have different colors. Rhododendrons, which are an important ornamental plant in the world due to their leaf characteristics and flower colors, have not yet been introduced to the ornamental plant sector in our country.

Rhododendron hirsutum was the first to be scientifically identified in rhododendrons. This species, also known as the Alpine rose, was discovered by the botanist Clusius (Charles l'Ecluse) in the 16th century and was taken to England from the Alps in 1656 for culture. *R. ponticum*, one of the natural species of our country, was discovered by Claes Alstoemer in the natural flora of Armenia (known as the Pontus Empire at that time) and Spain in Cadiz - Gibraltar in 1750 and taken to England in 1763. The German naturalist Pallas described four new species of rhododendrons in the flora of Eastern Europe and Asia between 1780 and 1796. Among these species, the species identified in 1793 is *R. flavum*, which is another natural species of our country and known as *R. luteum* today. In 1803, *R. caucasicum* species was taken to England as a gift from the Russian collector Count Pushkin (Anonymous, 2011a). Our other natural species, *R. smirnovii* and *R. ungernii*, were discovered by Baron Ungern-Sternberg in the Caucasus mountains in 1885. *R x sochadzeae*, one of the natural hybrid species of our country, was found in 1967; *R x rosifaciens*, *R x davisianum* and *R x filidactylis* were discovered by R. Milme in Tiryal Mountain (Murgul/ Artvin) in 1999 and were introduced to the scientific world by being defined at the molecular level (Milne et al., 1999; Avcı, 2004).

Today, rhododendrons are classified according to their structural and morphological, anatomical and chemical or physiological characteristics (Anşin and Özkan, 1993; Pulatkan, 2001; Sekergider, 2002; Anonymous, 2011b). According to this classification, rhododendrons,

Kingdom	: Plantae
Subkingdom	: Tracheobionta
Superdivision	: Spermatophyta
Division	: Magnoliophyta
Class	: Magnoliopsida
Subclas	: Dilleniidae
Order	: Ericales
Family	: Ericaceae
Genus	: *Rhododendron* L.

Rhododendron genus, which includes Turkey rhododendrons, is divided into 9 subgenus, 16 divisions and 56 subdivisions due to the differences between taxa. Within this taxonomy, *Rhododendron ponticum* L., *Rhododendron caucasicum* Pallas, *Rhododendron smirnovii* Trautv. and *Rhododendron ungernii* Trautv. species, which are natural rhododendrons of Turkey, are located in the Pontica subdivision of the Pontica section in the subgenus Hymenanthes while *Rhododendron luteum* Sweet is in the Pentanthera section of the Pentanthera subgenus. (Cullen 2005; Anonymous 2011c).

Rhododendrons naturally spread from sea level to alpine regions in the temperate climate zone of the Northern hemisphere. Almost 80% of its species are found in central and southeastern China, Pakistan, India, Nepal, Bhutan, Myanmar, the northwestern Himalayas of eastern Tibet, and Southeast Asia (Irving and Hebda, 1993). Rhododendrons, which naturally spread in the temperate and cool regions of the Himalayas, Japan, China, Malaysia, Northeast and West Asia and North America, are reported to be around 1000 to 1466 although the number of natural species varies according to different sources (Avcı, 2004; Rai. et al., 2013; e-floras, 2020).

Although almost all of the rhododendrons are seen in the northern hemisphere, *R. xenium* spreading at 3400 m altitude of Jaya Mountain in Papua New Guinea (Indonesia), *R. lochiae* spreading at 1500 m above sea level on

Australian Bellendenker mountain and *R. viriosum* in Australia are among the rhododendrons growing south of the equator. Rhododendrons, which grow in a wide area including the Alps in Europe, the Caucasus Mountains and the Himalayas in the east, also find distribution in Thailand, Vietnam, Malaysia and the Philippines in South East Asia. Rhododendrons, which are seen in the Indonesian islands by descending to the south, do not have any species that grow naturally in Africa and South America (Avcı, 2004). Rhododendrons, which show rich species diversity in the Himalayas, between central Nepal and Sikkim and the Yunnan and Sichuan regions in the east, are also common in the China-India Mountains, Korea, Japan and Taiwan (Anonymous, 2011d). Rhododendrons can live at altitudes close to sea level as well as they can go up to 5000 m above sea level in the Himalayan Mountains (Veteas et al., 2007). Nearly a hundred species of rhododendrons, which find a habitat at 4000 m in New Guinea, also grow on Mount Kinabalu, whose height exceeds 4000 meters, on the island of Borneo, located between southeast Asia and northern Australia (Stevens, 1985).

In Türkiye, rhododendrons start from Artvin in the east, spread along the entire Black Sea coast, in the sea-facing directions of the mountains and end on the northern slopes of the Istranca mountains in the west.

The Eastern Black Sea Region is the place where diversity is most intense on the basis of species. Except for the Black Sea Region, two species of rhododendrons (*R. ponticum* L. and *R. Luteum* Sweet.) were detected in small populations in Bursa (İznik), Yalova, Istanbul and Kırklareli, densely in Sakarya and Kocaeli in the Marmara Region. Also, *R. luteum* Sweet populations at two different points on the southern hillside of Kaz Mountains in Çanakkale (Altun et al., 2016).

Today, plants in the genus Rhododendron are extremely popular plants for outdoor use and as potted ornamental plants due to their characteristic features. Rhododendrons have become an important ornamental plant in the world with the colors of their flowers, plant form and leaf blade structure (Nelson, 2000). The aesthetic beauty of rhododendrons grown in nature has enabled these plants to be used in arrangements in today's modern gardens. In addition to the cultivated natural rhododendron species in urban landscape planning, rhododendron varieties developed by breeding studies and whose numbers are expressed in thousands today are widely used. Cultivated

rhododendrons are used in landscape planning for "aesthetic" and "functional" purposes. Rhododendrons used as an example plant, group or rock gardens as an aesthetic accent plant; functionally, it finds its place in outdoor organizations as a border element, curtain or fence plant (Harrison ve Jenny, 1997; Pulatkan, 2001).

When the habitat characteristics of rhododendrons in Turkey are examined, very special and limited areas emerge. The Black Sea Region is ecologically divided into coastal areas where the sea effect is seen and the high parts where the sea effect is not seen. In the coastal region, on the north-facing slopes of the mountains, there is a humid-temperate broad-leaved forest section in the lower part, which rises to approximately 1000 - 1500 meters, while in the upper parts there is a humid-cold coniferous forest section. The broad-leaved forest section is the richest area of our country in terms of plant species and communities. The dominant forest tree species in these areas are beech, chestnut, alder, linden, elm, maple, mountain ash, hornbeam and oak.

Coniferous trees dominate this region, completely different from the Black Sea coastal belt, due to the decrease in temperature with height as you approach from the hillside to the tops of the mountains. The forest trees in this area, which we call the Black Sea Mountain belt; spruce, fir, larch and yellow pine (Anonim 2007).

In the humid climate, forests generally form a mixed and stratified structure. In other words, trees, small trees, shrubs, vines, herbaceous plants, bulbous plants, fungi live together in the same area, one under the other or side by side, depending on their size. Rhododendrons constitute the most common small tree and shrub communities in these two ecoregions. Rhododendrons start from the seaside and reach the top of the forest; It forms dominant shrubs and small trees under forests (especially in sparse forests), in forest clearings. These regions have acidic soils preferred by rhododendrons. Rhododendrons show ideal growth in acidic (pH: 4.5-5.5), high moisture, rich in organic matter, deep and well-drained soils (Sekergider, 2002). Soil acidity is directly proportional to climatic conditions, especially precipitation. Soils in regions with high precipitation show acidic properties. In the eastern part of the Eastern Black Sea region, where rhododendrons grow intensively, the amount of precipitation exceeds 2000 mm. The distribution of precipitation according to the seasons is as important as the amount of precipitation. While summer precipitation rates

in this region reach 15% of the annual precipitation in most places, this rate exceeds 20% of the annual precipitation in Rize (Avcı, 2004). The precipitation rates in the regions where rhododendrons naturally spread in Turkey have made these soils acidic and therefore ideal environments for rhododendrons to grow.

Thanks to its morphological features such as flowers, leaves, habitus (general appearance), rhododendrons have never lost the interest of both breeders and researchers, since they were first described, and have always managed to keep their attention. With the aim of using rhododendrons, which have around 1000 (-1400) natural species in various countries of the world, as ornamental plants (indoor and outdoor) in countries such as America, England, Netherlands, Belgium and Japan, varieties with flowers in many different colors and shades have been developed. It has been reported that the number of these cultivars developed is 28.000 according to 2010 data (Spethmann et al., 2010), and around 30.000 according to 2017 data (Kondratoviès and Kondratoviès 2017). When these data are examined, it is seen that 2000 varieties were developed in a seven-year period. Therefore, these studies constitute an economically significant alternative business line (Sıralı ve Cınbırtoğlu, 2018). It is reported that almost 80% of European azaleas, which are in the same family as rhododendrons, are produced in East Flanders (Belgium), and approximately 30 million azaleas are produced in this region each year (Christiaens, 2014). *Rhododendron* and *Azalea* exports, which were 68 million dollars in 2015, reached 90 million 611 thousand dollars in 2019. Belgium is the leader in exports with a share of 44.91% and a value of 40 million 694 thousand dollars. Belgium is followed by the Netherlands (16.9 million dollars), Germany (8.4 million dollars), Canada (3.4 million dollars), America (3 million dollars) and Italy (2.4 million dollars). According to 2019 data, the countries with the highest imports of rhododendron and azalea are France (7.4 million dollars), the Netherlands (6 million dollars), Germany (5.2 million dollars), China (4.4 million dollars), Saudi Arabia (3.8 million dollars), Norway ($3.6 million dollars) and the United States ($3.4 million dollars). In the last 5 years between 2015-2019, rhododendron and azalea imports decreased by 7.32% in the world and amounted to 65 million 623 thousand dollars in 2019 (Anonymous 2020a). According to the 2018 data of the Netherlands, which is the leader in the world ornamental plants sector, the import and export of rhododendrons and azaleas are 4.7 million euros and 14 million euros, respectively (Anonymous 2020b).

In Dutch auctions, 9 million rhododendrons were sold at an average price of 1.44 euro/piece in 2018 (Anonymous 2020c, Altun, 2021).

Our country is an importer country in the rhododendron market. Turkey imported 277.208 rhododendrons and azaleas worth 742 thousand 331 dollars in 2019. This figure was 396 thousand 964 dollars for 171.628 rhododendrons and azaleas in 2021. In exports, 2.497 rhododendrons and azaleas were sold for 16 thousand 4 dollars in 2021 (TUIK, 2022).

Although there are 11 taxa rhododendrons in the natural flora of our country, which is among the importing countries in terms of rhododendrons and azaleas, these plants have not yet been introduced to the ornamental plants sector of our country. In our country, the wood of rhododendrons is used as fuel. In addition, R. ponticum species is used in the production of fiberboard and particleboard, while R. ponticum and R. luteum, which are located in the natural flora, are used in the production of honey known as mad honey in the region, contributing to the economy of the region producer (Sıralı ve Cınbırtoğlu, 2018).

Rhododendron species do not need direct sunlight, but they do not tolerate very intense shade either. Ideal light conditions are areas with partial sunlight and semi-shade. Evergreen species are more tolerant of shade conditions (Anonymous, 2011e).

Yellowing is seen on rhododendron leaves exposed to direct sun. If the sunlight is continuous, brown dead tissue will form on the leaves. (Sekergider, 2002). It has been observed that rhododendrons escape from direct sunlight by being inserted under forests or mountain slopes in their natural habitats.

Rhododendrons grow best in moderate temperatures. If the temperature is above 31°C for a long time, rhododendrons are adversely affected and damaged. Likewise, many species are damaged by shedding flower buds at temperatures below -25°C (Sekergider, 2002). R. smirnovii, one of the natural species of our country, can withstand temperatures up to -40°C without being damaged (Czekalski, 1998).

Rhododendrons, which have a high value as an ornamental plant, can be propagated by generative or vegetative methods as in many cultivated plants. While rhododendrons, which form abundant seeds with high germination ability, reproduce with seeds and underground stems in nature, they can also be reproduced by cuttings, grafting, dipping methods and tissue culture.

Reproduction of rhododendrons by seed is an easy and natural method. Rhododendrons are foreign pollinated plants. For this reason, intermediate species and hybrid individuals can be found in the natural flora as well as pure species (Altun, 2011; Altun et. al, 2016). Seed propagation studies are a more suitable method for breeding studies to obtain new hybrid plants or for studies to obtain rootstocks in grafting. Since there may be genetic expansion, seedling production from seed is not a commercially viable method. In vegetative propagation, parts of perennial plants such as roots, stems, tubers, rhizomes, shoots, leaves are used. Rhododendrons are generally propagated by cutting, grafting, layering and tissue culture (Sekergider, 2002).

1. Production by Seed

1.1. Collecting Seeds and Sowing

The first thing to know about the propagation of rhododendrons with seeds is that the seeds do not need chilling and they need light for germination. After the seed capsules of the rhododendrons turn brown, before the capsules are completely opened and the seeds are scattered around (Figure 1), the seed capsules should be taken and placed in a clean and dry cloth bag and brought to the sowing site.

Figure 1. Rhododendron seed capsules (*Original: B. ALTUN*)

Seed capsules should be placed in large containers in a dry environment at room conditions and the capsules should be opened completely. Seeds should be extracted from the opened capsules and made ready for sowing (Figure 2).

Figure 2. Seed capsules and seeds of R. caucasicum Pallas species (*Original: B. ALTUN*)

Rhododendrons have rather small seeds. In a study conducted by Altun (2011) the seed weights of 1000 seeds of Turkish rhododendron species were determined and it was reported that the average seed weight was between 0.142 g and 0.067 g (Table 1).

Table 1. 1000 grain weights of Rhododendron seeds

SPECIES	**Average (g)**
Rhododendron ponticum L.	0.073
Rhododendron luteum Sweet	0.142
Rhododendron smirnovii Trautv.	0.076
Rhododendron ungernii Trautv.	0.071
Rhododendron caucasicum Pallas	0.067

2. Sowing the seeds

Very small seeds should be sowed superficially on acidic peat, preferably under fogging conditions. Care should be taken to ensure that the seeds do not clump together and disperse homogeneously on the surface while sowing seeds. Irrigation should be done carefully by misting with lime-free water to prevent the seeds from clumping together. In addition, the environment should not be allowed to dry out and the environment should be kept constantly moist. Therefore, sowing under the fogging unit provides a great advantage. When the ambient temperature is kept around 20°C, it can be seen that the sown seeds germinate and emerge on the soil surface within two weeks, depending on the species (Figure 3).

Figure 3. Germinated and hatched rhododendron seeds (*Original: B. ALTUN)*

After the real leaves appear on the rhododendron plantlets that germinate and emerge on the soil surface, they should be staggered into pots and grown in a humid environment. Irrigation should be done with lime-free water if possible.

2. Propagation by cutting

2.1. Receiving, preparation and storage of cuttings

Rhododendrons are propagate by tip cuttings. In rooting process, each type may show different reactions according to different cutting periods and

different applications. In a study conducted by Altun (2011) the rooting rates of Turkish *Rhododendron* species according to cutting time, planting environment and applied IBA dose are given in Table 2.

Table 2. Rooting rates of Turkish rhododendron species according to cutting time, planting medium and applied IBA dose

Species	Cutting receiving period	Planting medium	IBA (ppm)	Rooting rate (%)
R. ponticum L.	October	Acidic Peat	8000	86.00
R. luteum Sweet	October	Perlite	8000	95.00
R. caucasicum Pallas	November	Perlite	2000	41.60
R. smirnovii Trautv.	August	Perlite	0	73.33
R. ungernii Trautv.	August	Acidic Peat	2000	21.00

In order to minimize water loss, especially in evergreen species or summer cuttings of deciduous species, half of the crown leaf should be left and all other leaves should be removed (Figures 4 and 5). If the cuttings prepared in this way will not be planted immediately, they should be tied in bundles and placed in foam containers lined with moist newspaper and kept in cold conditions until planting time by placing an ice pack on them (Figure 6).

Figure 4. Rhododendron tip shoots (*Original: B. ALTUN*)

Figure 5. Prepared rhododendron tip cuttings (*Original: B. ALTUN*)

Figure 6. Cuttings packed in foam containers in bundles (*Original: B. ALTUN*)

2.2. Preparation of Growth Regulatory Substance (IBA)

Synthetic auxins such as Indole-3-Butyric Acid (IBA) and naphthalene acetic acid (NAA) can be used to increase the rooting rate in the propagation of rhododendrons by cuttings (Almeida et al., 2003; Strzelecka, 2007; Altun, 2011). The 100 ml and 2000 ppm dose of IBA, which can be supplied in powder form, is prepared as follows: The tare of the container you will weigh is taken on a precision scale. 200 mg of powdered IBA is poured into the weighing container and weighed. 200 mg of IBA is dissolved in 96% pure alcohol (ethyl alcohol) and completed with pure alcohol, with a total alcohol content of 50 ml. The solution is made ready for use by adding 50 ml of distilled water to the IBA and pure alcohol solution.

2.3. Planting the cuttings

The cuttings, brought to the rooting greenhouse where the planting will be made, should be cut straight about 0.5 cm in order to reach the living tissue from the bottom sides. The bottom parts of the prepared cuttings should be kept in the prepared IBA solution for 5 seconds. Cuttings taken from IBA should be planted in a temperature (under-heating) and humidity-controlled (under fogging) rooting environment, in such a way that 2/3 of the cuttings remain in the environment and their leaves do not come into contact with each other

(Figure 7). The temperature of the rooting medium should be kept around 24°C. The fogging unit should be adjusted so that the leaves of the cuttings and the humidity of the rooting environment do not dry out.

Figure 7. Rhododendron cuttings planted in rooting medium (*Original: B. ALTUN*)

In the cuttings that will be checked regularly, taking into account the ambient humidity and the presence of leaves, weekly pesticides should be applied against fungal diseases. Cuttings taken from rhododendron species do not root easily and in a short time like many other plants. After rooting occurs in the cuttings that remain in the planting environment for about 220 days, the rooted cuttings should be carefully removed and taken into separate pots. The pots should be filled with a mixture of forest soil and acidic peat (3:1) and planting should be done in this medium. The pots should be watered regularly with a lime-free water in a place with indirect light and the seedlings should be grown in this environment until they are transplanted to their real place.

3. Production by air layering

Air layering is one of the methods used in vegetative propagation of some plants. This method, which is very easy to apply, can also be used in outdoor ornamental plants, some fruit species (fig, mulberry, etc.) and indoor ornamental plants such as *Ficus* spp. The biggest advantage is that seedlings can be obtained without any damage to the mother plant and it saves a

significant amount of time for the producer (Tiwari and Chauan, 2006). This method allows to obtain seedlings of maturity and size that can be planted in the garden if rooting occurs, since the branch of the plant to which air leyering is applied is of a certain age. Air layering is a method that is simple to apply, requires little material, and obtains relatively large (about 1 m tall) rooted plants (Brennan and Mudge, 1998).

In order to make air layering, firstly the shoots of the appropriate size should be selected and the layering process should be done approximately 50 cm below the shoot tip. When choosing the branches to be air layering, a sample shoots that can be used in the garden after rooting should be selected. The selected area should be cleaned of branches and leaves, and 1-2 cm vertical scratches should be created on three sides of the trunk diameter axis, on the bark part, with a sharp knife. IBA solution prepared at a dose of 500 ppm can be sprayed on these areas. After applying IBA, the black plastic mulch prepared in the form of a strip should be wrapped around this wound area and adhered with paper tape from the bottom to form a gap. This gap should be filled with acidic peat and the top of the plastic mulch should be covered with paper tape again (Figure 8). Although the applications vary according to the species, they can be done in autumn or spring. Plants that remain this way for about 1 year should be cut from under the dipped part at the end of the year and left in water for at least 24 hours to extract water. Then, the mulch should be removed and the seedlings should be planted in the environment prepared for planting and life water should be given.

Figure 8. *Rhododendron* shoot made with air layering (*Original: B. ALTUN*)

RESULTS

Rhododendrons are plants that have always managed to attract the attention of both researchers and producers since they were first described. Over time, they have become an important trade material by taking their place in the economy. Although 11 taxa and many genotypes are naturally distributed in our country, our country is the importer country for these plants. It can be said that the main reasons for this are that the propagation of these plants is not easy like many plants and the propagation techniques are not well known. With this compilation study, it is aimed to introduce rhododendrons and to give information about production techniques that can be easily applied by everyone.

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CHAPTER 9

INVESTIGATION OF THE KNOWLEDGE LEVELS, ATTITUDES, AND BEHAVIORS OF GRAIN MAIZE PRODUCERS REGARDING PESTICIDE: CASE OF KONYA PROVINCE, YUNAK DISTRICT

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INTRODUCTION

The rapid increase in the world population in the last century has led to increased industrial production, especially food production, and urbanization rates. With the rapid population growth, it has become inevitable to get the highest yield from the unit area in agricultural areas to meet the food needs of people. The increase in urbanization rates to meet the housing needs of people may indirectly cause a decrease in agricultural areas. Various agricultural practices are carried out to optimize the scarce resources in nature and increase the yield per unit area. Pesticide applications are carried out to increase the yield of agricultural products obtained from the unit area. Pesticide use is part of these practices. In the literature, it has been reported that crop losses may occur if pesticides are not used in the plant production process (Tanrivermiş 2000; Uzundumlu 2005; Tiryaki et al. 2010). It is implemented to reduce its environmental impact (Aslan et al., 2020; Günay, 2020). Today, chemical control tools have a positive effect on production efficiency. However, the unconscious use of pesticides negatively affects human health over time and causes significant damage to the sustainability of the ecosystem (Anonymous, 2022a; Anonymous, 2022b).

One of Turkey's products subject to pesticide applications is the grain maize plant, which has an important share in agricultural production. Konya province is among the leading provinces in grain maize production in Turkey. As of 2021, 7582370 (de) grain maize has been planted in Turkey, and the maximum production amount reached 6750000 tons. As seen in Table 1, grain maize production in Konya in 2021 was 1241821 (de), and the maximum cultivation area was reached. Grain maize production in Konya reached the highest annual production in 2021, with 1261475 tons. It is observed that there have been fluctuations in grain maize cultivation areas and production amounts in the last ten years on a country and regional basis.

Table 1. Maize cultivation area and production amount in Turkey.

Year	Konya				Turkey			
	Cultivation Area (de)	Ratio (%)	Production (ton)	Ratio (%)	Cultivation Area (de)	Ratio (%)	Production (ton)	Ratio (%)
2011	230934	2.97	159858	2.04	5890000	8.16	4200000	6.53
2012	382539	4.93	312059	3.99	6226094	8.62	4600000	7.15
2013	341310	4.40	353552	4.52	6599980	9.14	5900000	9.17
2014	381002	4.91	382099	4.89	6586450	9.12	5950000	9.25
2015	548861	7.07	558190	7.14	6881699	9.53	6400000	9.95
2016	635269	8.19	638300	8.17	6800192	9.42	6400000	9.95
2017	637972	8.22	621884	7.96	6390844	8.85	5900000	9.17
2018	1074626	13.8	1104538	14.1	5919003	8.20	5700000	8.86
2019	1245353	16.0	1345064	17.2	6388287	8.85	6000000	9.33
2020	1033998	13.3	1070626	13.7	6916324	9.58	6500000	10.1
2021	1241821	16.0	1261475	16.1	7582370	10.5	6750000	10.4

Source: Turkish Statistical Institute (TUIK) (Anonymous, 2022c).

There are scientific studies on the use of pesticides in herbal production. Özkan et al., (2000) examined the attitudes and behaviors of farmers regarding the use of pesticides in the production of citrus fruits. Within the scope of the study, the survey studies conducted in Finike, Kemer, Kumluca, Manavgat, and Serik districts are summarized as follows: 74.15% of the producers apply pesticides when diseases and pests are observed. 49.7% of the manufacturers determine the agricultural drug selection in line with their own experience, and 42.78% determine the chemical drug selection according to the dealer's recommendation. 70.4% of the farmers stated that chemical pesticides left residue on the plant produced, and 96.8% noted that excessive use of chemicals pollutes nature. Çelik and Karakaya (2017) conducted a study on the evaluation and economic analysis of knowledge, attitudes, and behaviors in the use of pesticides in the Adaklı district of Bingöl province, where apple production is made. Within the scope of the research, 59.9% of the producers paid attention to the effectiveness of the agricultural drug in selecting chemical pesticides. In comparison, 30.4% stated that the recommendations of the technical staff of the provincial and district directorates of agriculture were effective in selecting chemical pesticides. 50% of the farmers store chemical drugs in barns and shelters, 35% in a special cupboard, and 15% in any part of the house. 50% of the farmers destroy their empty chemical drug boxes or bags by burning them

after they are stored in one place. While 30% of them throw it in the garbage cans, 20% say they throw it anywhere in the house's garden. Alben and Boz (2014) investigated the use of pesticides by farmers in the Türkoğlu District of Kahramanmaraş Province. Researchers reported that most regional producers know pesticides harm the environment and nature, and 75.5% receive consultancy services on pesticides. In addition, it was determined that the rate of use of pesticides was 40%, according to the advisors of the producers, and 33.3% of the producers acted on their own experiences. The study concluded that state bodies and consultants have important duties to ensure conscious pesticide use. Şahin et al., (2010) investigated the incidence of pesticide poisoning among apple growers in the Eğirdir and Gelendost districts of Isparta province. They evaluated the knowledge, attitudes, and behaviors of individuals who sprayed pesticides. In their results, the researchers reported that the rate of pesticide poisoning in the last year was 1.3%. In addition, the researchers determined that personal precautions were not considered during the spraying, the pesticide dosage was used outside the normal, and the time required to wait after the spraying was not followed. Inan and Boyraz (2002) examined the behavior of producers in the agricultural struggle practices in Konya. In the research findings, it was determined that the education level of the farmers was largely low. In their studies, the researchers reported that the producers used more pesticides than the required dose in agricultural spraying. They found that the awareness of this issue was relatively low. Akar and Tiryaki (2018) investigated farmers' level of education and knowledge and environmental sensitivities in pesticide use and selection in Antalya Province. Researchers reported that approximately 58.2% of producers have a high level of ecological awareness. In addition, it was stated that 12.7% of the farmers did not pay attention to the time between spraying and harvesting, 44.4% of them emptied the excess sprayed water to one side of the garden, 26% of them threw the empty pesticide boxes in the trash, and 8.5% of them threw them into the environment. Günay (2020) researched the knowledge levels, attitudes, and behaviors of pesticide use of capia pepper producers. As a result of the research, it was revealed that the farmers were familiar with the weeds, insects, and fungi that did or did not harm the capia pepper. In addition, the researcher determined that capia pepper farmers generally prefer Chambers of Agriculture and Specialty Pharmaceutical Dealers in the supply of pesticides, they use tractors

and back pulverizes as the application tool, and the use of scale in pesticide application, the packaging and wastewater disposal methods of the farmers after spraying are in a way that harms the environment.

This study aimed to determine the pesticide use in grain maize production in the Yunak district of Konya province and the attitudes and behaviors of producers, as well as their knowledge levels about pesticide use. The model structure designed in the study; was prepared by benefiting from the questionnaire study developed by Günay, (2020). The questionnaire prepared by the researcher was adapted to the regional conditions. Based on demographic variables in the model, it was investigated whether there were statistically significant differences in pesticide use awareness, attitudes and behaviors on pesticide use, and agricultural pesticide supply characteristics scales.

MATERIAL and METHOD

Material

The study sample consists of the questionnaire data made to the farmers who produce grain maize and use pesticides in the Yunak district of Konya rural. The questionnaire study was conducted face-to-face. The sample size of the study; The power of the test was 0.80, the effect size was 0.5, and the type 1 error level was 0.05, and it was determined as 100 people in the light of the power analysis data. However, 80 questionnaires were evaluated.

The questions in the survey were prepared by benefiting from the study by Günay (2000). The questionnaire form used in the study consists of five parts. In the first part, there are questions to determine the demographic characteristics of the participants. In the second part, there are questions arranged according to a 5-point Likert-type scale to determine the awareness level of the farmers participating in the survey about pesticide use. The third part consists of 26 statements arranged according to a 5-point Likert-type scale to determine the attitudes and behaviors of grain maize producers regarding pesticide use. The questions in the fourth part were set to determine the pesticide supply characteristics of grain maize producers. The fifth part of the survey consists of questions about the grain maize production and other production areas of the farmers participating.

Method

Within the scope of the study, the profile of the farmer producing grain maize in the Yunak district of Konya province was examined, and its demographic and general characteristics were determined. Awareness levels of farmers about pesticide use are discussed under the headings of consumer health, farmer health, and environmental problems before, during, and after spraying. Attitudes and behaviors of grain maize producers on pesticide use were determined. Agrochemical supply characteristics of herbicide, fungicide, and insecticide types were determined. The study's analysis was performed with the SPSS (Statistical Package for the Social Sciences) 26.0 statistical program.

RESULTS

Socio-Economic and Demographic Characteristics of Farmers Producing Grain Maize

In the result section of this research, first of all, information about demographic variables was included. Demographic characteristics of grain maize producers were determined as gender, age, education level, main sources of income, experience information, and the number of people in their households. In addition to these, some questions about agricultural incomes were included.

According to Table 2, 100% of the grain maize producers surveyed are male. The average age of the producers was calculated as 44 years. In addition, the youngest farmer among the producers is 20 years old, and the oldest farmer is 65 years old. When the age distributions are examined, it has been determined that 5% of the grain maize producers are between the ages of 15 and 24, 17.5% are between the ages of 25 and 34, 38.8% are between the ages of 35 and 49, and 38.8% are between the ages of 50 and 64. 3.8% of grain maize producers are primary school graduates, 10% are secondary school graduates, 72.5% are high school graduates, 10% are college graduates, and 3.8% are university graduates. Within the scope of the study, the number of people living in their households was asked of the farmers, and they were grouped in two intervals. The average number of people in the households of the producers was calculated as 5.13. The number of people living in the households of grain maize producers is at least two and, at most, nine. While the average farming experience of grain maize producers was 19.6 years, the average grain maize

production experience was calculated as 9.6 years. It has been determined that approximately 97.5% of the farmers' land assets are owned property. It has been determined that 57.5% of the farmers have social security, 42.5% do not, and 97.5% are not cooperative members.

Table 2. Demographic variables of grain maize producers.

Variables		Frequency (n)	Percent (%)
Gender	Female	0	0
	Male	80	100
Age	15-24	4	5
	25-34	14	17.5
	35-49	31	38.8
	50-64	31	38.8
Marital Status	Married	65	81.3
	Single	15	18.8
Education Status	Primary School	3	3.8
	Middle School	8	10
	High School	58	72.5
	College	8	10.0
	University	3	3.8
Number of Person in Household	0-4	32	40
	5-10	48	60
Social Security	No Social Security	34	42.5
	Bağ-Kur	46	57.5
Cooperative Membership	Yes	2	2.5
	No	78	97.5

Information on Production Status of Grain Maize Producers

Information about grain maize production in the Yunak district of Konya province is given in Table 3. According to the analysis, the average size of the fields in the study region that produce grain maize is 174.8 decares, the average number of parcels is 1.0125 parcels, and the average grain maize yield is 1487.3375 kg.

Table 4 shows the average values of the determined annual agricultural income-expenditure and grain maize income-expenditure levels during the research period. Accordingly, the annual agricultural income level was

determined to be 937450 TL on average. The producers' estimated annual agricultural expenditure level in the last period was determined as 386875 TL on average. The producers' estimated annual grain maize income level in the last period was determined as 779075 TL on average, and the annual grain maize expenditure level of the last period estimation was determined as 357087.5 TL on average.

Table 3. Information on production status of grain maize producers

Variables	Min.	Max.	Mean
Grain maize produced area (own place- icar)	18 da	420 da	174.8 da
Number of parcels produced in grain maize	1 Parcel	5 Parcels	1.0125 Parcel
Maize average yield/ decare	1200 kg	1900 kg	1487.3375 kg

Table 4. Agricultural income levels of farmers

Variables	Mean
Approximate annual agricultural income level in the last period	937450 TL
Approximate annual agricultural expenses level in the last period	386875 TL
Approximate annual grain maize income level in the last period	779075 TL
Approximate annual grain maize expense level in the last period	357087.5 TL

Pesticide Supply Characteristics of Grain Maize Producers

Table 5 shows the recognition status of grain maize producers for insects that harm maize, insects that do not harm maize, weeds that do not harm maize, and fungi that harm maize. Grain maize producers were made 5-point Likert-Scale judgments about the research (5= I definitely know… 1= I definitely not). 73.8% of the farmers within the scope of the study were undecided whether to recognize the insects that harm the grain maize and 47.5% of the producers are undecided whether to recognize the insects that do not harm the grain maize. 68.6% of the farmers were undecided whether to recognize weeds that harm grain maize and 50% of them recognize weeds that harm grain maize. Fungi that damage grain maize are recognized by 52.5% of our farmers. However, 31.3% of the farmers were undecided whether to recognize the fungi that harm the grain maize. According to the answers given by the producers, it can be interpreted that the producers of grain maize are familiar with the insects, weeds and fungi that damage the maize.

Table 5. Pesticide supply characteristics of grain maize producers (%)

Variables	Definitely Know	Know	Undecided	Don' t Know	Definitely Don' t Know
1. Insects that damage grain maize	1.3	73.8	15	8.8	1.3
2. Insects that do not harm grain maize	1.3	26.3	47.5	23.8	1.3
3. Damage weeds that grain maize	13.8	68.8	15	2.5	.
4. Weeds that do not harm grain maize	2.5	35	50	12.5	.
5. Fungi that damage grain maize	3.8	52.5	31.3	11.3	1.3

Table 6. Pesticide type selection characteristics of the farmers within the scope of the study

Variables	Herbicide %	Fungicide %	Insecticide %
According to my own experience	55	56.6	55
Consulting to neighbor	30	30	31.3
In consultation with the provincial-district agricultural directorates	10	7.5	10
Based on recommendations from other agricultural organizations	7.5	7.5	7.5
Based on recommendations from agricultural pesticide dealers	57.5	60	53.8
Other	0	0	0

In this part of the study, the opinions of grain maize producers on pesticides were examined under three main headings: Herbicide (herbicide), Fungicide (fungicide), and Insecticide (insecticide). Information on the factors affecting the pesticide applications of grain maize producers is given in Table 6. It has been determined that the farmers in the research region have determined their choices for all three pesticide types according to the recommendations of the pesticide dealers (57.5%, 60%, 53.8%) and according to my own experience (55%, 56.6%, 55%).

Information on the farmers' places of supply of pesticides is given in Table 7. The most preferred option in the herbicide type was private pesticide dealers, with a value of 78.8%, chambers of agriculture, 17.5%, and agricultural

credit cooperatives, 16.3. The most preferred option for fungicide and insecticide types was private pesticide dealers, agriculture chambers, and agricultural credit cooperatives.

Table 7. Pesticide supply places of farmers

Variables	Herbicide %	Fungicide %	Insecticide %
Private pesticide dealers	78.8	77.5	78.8
Agricultural credit cooperatives	16.3	16.3	16.3
Chambers of agriculture	17.5	18.8	17.5
Other agricultural organizations	3.8	3.8	3.8
Other	0	0	0

Table 8. Factors effective in farmers' agricultural spraying decision

Variables	Herbicide %	Fungicide %	Insecticide %
Without seeing (precautionary purpose)	31.3	32.5	28.8
When the disease or pest is first seen in the field	33.8	35	36.3
When the degree of pest infestation intensifies	12.5	11.3	12.5
When the early warning comes	6.3	3.8	5
Based on recommendations from agricultural organizations	8.8	8.8	10
Based on recommendations from pesticide dealers	43.8	46.3	47.5
Consulting the neighboring farmer	11.3	10	10
Other	0	0	0

Table 8 contains information on the factors that are effective in the agricultural spraying decision of the farmers. Accordingly, it is seen that similar responses are obtained for each type of pesticide. It has been revealed that the farmers make decisions based on the recommendations of the pesticide dealers about spraying. In addition, it has also been determined that the farmers apply pesticides as soon as they see the disease or pest in the field without seeing them for preventive purposes.

In Table 9, information on the pesticide use doses of the farmers is given. It was determined that the farmers adjusted the dosage of pesticides according to the pesticide package insert (81.3%) in herbicide, fungicide, and insecticide applications. It was determined that some of them took into account the recommendations of exceptional agricultural engineers in the applications of Herbicides (18.8%), Fungicides (18.8%), and insecticides (20%) and made dose adjustments according to their own experience.

Table 9. Factors effective in determining the pesticide dose for farmers

Variables	Herbicide %	Fungicide %	Insecticide %
According to his/her own experience	13.8	12.5	13.8
According to his neighbor's advice	5	3.8	3.8
Recommendations of provincial-district agricultural directorates	7.5	7.5	6.3
Special agriculture engineer advice	18.8	18.8	20
According to the pesticide package insert	81.3	81.3	81.3
Other	.	.	.

Table 10. Tools and equipment used in dosing pesticide

Variables	Herbicide %	Fungicide %	Insecticide %
Using scale	81.3	77.5	81.3
Using the pest cap	27.5	31.3	27.5
Using a glass	8.8	8.8	8.8
Eyeball estimate	1.3	1.3	1.3
Other	0	0	0

Table 11. Elements used by farmers in pesticide application

Variables	Herbicide %	Fungicide %	Insecticide %
Hand pulverizator	0	0	0
Back pulverizator	0	0	0
Tractor pulverizator	100	100	100
Other	0	0	0

When the tools and equipment used by the grain maize producers to determine the pesticide dose are examined, it is stated that they determine the pesticide dose by using scales, using a pesticide cap, using a glass, and finally by eye decision, in the applications performed in all three pesticide groups, as can be seen in Table 10. According to the results in Table 11, it was determined that all of the farmers applied pesticides with tractor sprayers.

Table 12. Disposal methods of empty pesticide packages

Variables	Herbicide %	Fungicide %	Insecticide %
Dispose to litter	41.3	41.3	42.5
Dispose to the edge of the field	15	15	16.3
Burying under the ground	1.3	1.3	0
Burning	71.3	73.8	72.5
Dispose to the riverside	1.3	1.3	1.3
Other	0	0	0

Information on the farmers' methods of destroying empty packages after spraying is given in Table 12. Herbicide-Fungicide-Insecticide packages are destroyed by burning (71.3%, 73.8%, 72.5%), throwing away (41.3%, 41.3%, 42.5%) and throwing them to the side of the field (15%, 15%, 16.3%). Table 13 contains information about the places where washing water is poured in pesticide applications. Farmers stated that in all three pesticide groups (98.8%, 98.8%, 98.8%), they poured washing water on the field or the edge of the field.

Table 13. Where farmers spill wash water

Variables	Herbicide	Fungicide	Insecticide
	%	%	%
To the field/the field edge	98.8	98.8	98.8
Tree bottoms	0	0	0
By the stream/the stream edge	1.3	1.3	1.3
Other	0	0	0

Table 14 shows the distribution of farmers' pesticide storage locations. The results show that the farmers mostly store pesticides in warehouses (Herbicide: 65%- Fungicide 66.8%- Insecticide 66.3%) and garages (Herbicide: 36.3%- Fungicide 36.3%- Insecticide 35%). Some farmers reported keeping it in the open-air environment (Herbicide: 20%- Fungicide 20%- Insecticide

21.3%). According to the results in Table 15, the farmers throw away the expired or unused pesticide (Herbicide: 87.5%- Fungicide 87.5%- Insecticide 86.3%), burn them (Herbicide: 16.3%- Fungicide 16.3%- Insecticide 16.3%) and pesticide dealers. (Herbicide: 11.3%- Fungicide 11.3%- Insecticide 11.3%) were determined.

Table 14. Farmers' pesticides storage areas

Variables	Herbicide %	Fungicide %	Insecticide %
Warehouse	65	66.8	66.3
Cellar	3.8	2.5	1.3
House	0	0	0
Garage	36.3	36.3	35
Open-air	20	20	21.3
Other	0	0	0

Table 15. Opinions of farmers on outdated or unused pesticide

Variables	Herbicide %	Fungicide %	Insecticide %
Dispose to litter	87.5	87.5	86.3
Dispose to the edge of the field	5	5	5
Burying under the ground	0	0	0
Burning	16.3	16.3	16.3
Dispose to the riverside	0	0	0
Giving to pesticide dealer	11.3	11.3	11.3
Other	0	0	0

Table 16. Factors considered while purchasing pesticide

Variables	Herbicide %	Fungicide %	Insecticide %
Cheap price	56.3	56.3	56.3
Being the most effective pesticide	82.5	80	80
Have a friend's recommendation	16.3	18.8	17.5
Recommendation of the provincial, district directorate of agriculture	6.3	7.5	8.8
Pesticide dealer advice	33.8	32.5	32.5
Other	0	0	0

The factors that farmers pay attention to when purchasing pesticides are summarized in Table 16. Farmers drew attention to the necessity of high efficiency of pesticides (Herbicide: 82.5%- Fungus 80%- Insecticide 80%). In addition, the price is low (Herbicide: 56.3%- Fungicide 56.3%- Insecticide 56.3%), dealer recommendation (Herbicide: 33.8%- Fungicide 32.5%- Insecticide 32.5%), and familiar recommendation (Herbicide: % 16.3- Fungicide 18.8%- Insecticide 17.5%) were among the effective factors in pesticide applications for farmers

Table 17. Reasons for farmers to use out of recommended pesticide

Variables	Herbicide %	Fungicide %	Insecticide %
Insufficient recommendation	85	86.3	86.3
Interest in new pesticide	8.8	8.8	7.5
Being influenced by pesticide dealers	6.3	6.3	6.3
Influence from other farmers around	17.5	17.5	17.5
Other	1.3	1.3	1.3

Table 18. The efficiency of the pesticide used by farmers

Variables	Herbicide %	Fungicide %	Insecticide %
Very efficient	20	18.3	20
Effective	76.3	76.3	71.3
Less effective	5	5	8.8
No effect	0	0	0
Makes it worse	0	0	0

Table 17 shows the reasons for farmers to use out of recommended pesticides. It was determined that the farmers mostly used out of recommended pesticides in cases where the recommended pesticide was insufficient (Herbicide: 85% - Fungicide 86.3% - Insecticide 86.3%). In addition, the factors that are effective in the use of out of recommended pesticides are stated as follows: Being affected by the farmers in the environment (Herbicide: 17.5%- Fungicide 17.5%- Insecticide 17.5%), being curious about new pesticide (Herbicide: 8.8%- Fungicide 8.8%- Insecticide 7.5%) and pesticide dealers (Herbicide: 6.3%- Fungus 6.3%- Insecticide 6.3%). The evaluations of

the maize producers regarding the effectiveness of the pesticides they use are given in Table 18. Majority of the farmers reported that pesticides were effective (Herbicide: 76.3%- Fungicide 76.3%- Insecticide 71.3%) and some of them reported that they were very effective (Herbicide: 20%- Fungicide 18.3%- Insecticide 20%).

Table 19. Hazard dimensions of pesticide application in terms of farmer and environmental health

Variables	Farmer Health			Environmental Health		
	Herb. %	Fung. %	Insect. %	Herb. %	Fung. %	Insect. %
Extremely dangerous	87.5	87.5	90	93.8	91.3	92.5
Moderately dangerous	8.8	8.8	6.3	5	7.5	7.5
Slightly dangerous	3.8	2.5	1.3	2.5	1.3	1.3
Least dangerous	1.3	1.3	2.5	0	0	0
No effect	0	0	0	0	0	0

The opinions of grain producers about the danger dimensions in terms of farmer and environmental health in pesticide applications were taken. As can be seen in Table 19, farmers found pesticides extremely dangerous in terms of both farmer's health (Herbicide: 87.5%- Fungicide: 87.5%- Insecticide: 90%) and environmental health (Herbicide: 93.8%- Fungicide: 91.3%- Insecticide: 92.5%).

Awareness Levels of Farmers on the Use of Pesticides

In this part of the research, the answers given to the questionnaire questions about the awareness of the farmers who produce grain maize about pesticide applications are summarized. First, farmers' opinions about consumer, farmer and environmental health before pesticide application are given. Table 20 shows the opinions of the farmers about consumer, farmer, and environmental health before pesticide application. It was determined that the farmers gave average answers rather than positive or negative judgments about consumer health before spraying. Most of the farmers stated that they attach importance to protective clothing, equipment uses and warnings on the packaging before spraying. Views of farmers on environmental health before spraying are partially similar to their views on consumer health. In the general

evaluation, it can be interpreted that the farmers are more sensitive about environmental health before spraying.

Table 20. Opinions of farmers on consumer, farmer, and environmental health before pesticide application

	Strongly Disagree	Disagree	Neither agree nor disagree	Agree	Strongly Agree
Consumer health before spraying (%)					
1. I use whatever pesticide is necessary to fight the disease in grain maize before spraying; the health of those who consume grain maize after harvest comes later for me.	12.5	20	25	33.8	8.8
2. Before spraying, I use pesticides as often as necessary to combat the disease in grain maize, the health of those who will consume grain maize after harvest comes later for me.	13.8	20	27.5	30	8.8
3. When choosing a pesticide before spraying, I look at whether it is effective in the disease in grain maize; after harvest, the health of people who consume maize grain comes later for me.	15	21.3	26.3	27.5	10
4. It is important for me to fight the disease in grain maize; I don't care about the rest.	10	23.8	25	30	11.3
Farmer health before spraying (%)					
1. I always wear protective clothing (gloves, overalls, etc.) before pesticide app.	7.5	10	6.3	48.8	27.5
2. I always wear protective equipment (mask, goggles, etc.) before the pesticide app.	7.5	15	3.8	45	28.7
3. I read the warnings on the pesticide before the application.	7.5	12.5	6.3	46.3	26.3
Environment health before the spraying (%)					
1. It is important for me to fight the disease in grain maize; giving the least pesticide to the soil comes later for me.	15	27.5	22.5	27.5	7.5
2. It is important for me to fight the disease in grain maize; giving the least pesticide to the water comes later for me.	13.8	30	21.3	30	5

3. For me, it is important to fight the disease in grain maize; giving the least pesticide to the air comes later for me.	15	31.3	20	28.7	5
4. For me, it is important to throw how much pesticide is needed on grain maize; environmental pollution is in the background.	15	30	22.5	27.5	5

Table 21. Opinions of farmers on consumer, farmer, and environmental health during pesticide application

	Strongly Disagree	**Disagree**	**Neither agree nor disagree**	**Agree**	**Strongly Agree**
Consumer health during spraying					
1. I don't leave a long/enough time between spraying and harvest; I collect.	50	20	10	13.8	6.3
2. I use a lot of pesticide until the disease in the maize is over.	11.3	32.5	15	35	6.3
3. It is important to get rid of the disease in the grain maize when spraying; it is secondary for me that the people who will eat this product are sick.	16.3	16.3	36.3	26.3	5
Farmer health during spraying					
1. I do not care about my health during the application.	76.3	20	1.3	2.5	.
2. I am affected by the pesticide (eye redness, cough, dizziness, etc.)	75	13.8	2.5	5	3.8
Environmental health during spraying					
1. My priority during spraying is to eradicate the disease in grain maize; polluting the soil, water, and air comes later for me.	6.3	23.8	35	33.8	1.3
2. During the application, I used more pesticide as needed.	3.8	17.5	33.8	42.5	2.5
3. I apply the pesticide as often as necessary during the application.	5	13.8	35	43.8	2.5
4. I do the spraying slowly during spraying.	7.5	11.3	45	32.5	3.8

Table 21 shows farmers' opinions about consumer, farmer, and environmental health during agricultural spraying. About 50% of the farmers reported leaving a long/sufficient time between pesticide application and harvest. It is known that attitude, as mentioned earlier, is very important in

terms of consumer health. The farmers' priority is to get rid of the diseases when they disease is encountered or when a pesticide application is required. It has been determined that approximately 76.3% of the farmers give importance to their health during spraying, and 75% are physically affected by pesticides. When the views on environmental health during spraying are examined, it is seen that the agricultural product, which is the subject of production, is the focal point. Farmers apply the required dose of pesticides and repeat the spraying in a way that considers the plant's health.

Table 22. Opinions of farmers on consumer, farmer, and environmental health after pesticide application

	Strongly Disagree	**Disagree**	**Neither agree nor disagree**	**Agree**	**Strongly Agree**
Consumer health after spraying					
1. I think, there is a lot of pesticide residue in the grain maize I sell after harvest.	6.3	20	52.5	21.3	.
2. I am concerned about the health of consumers because of the residue in the maize, I sell after harvest.	7.5	40	41.3	10	1.3
Farmer health after spraying					
1. I feel bad after spraying.	60	32.5	5	1.3	1.3
2. I get sick after spraying.	62.5	28.7	2.5	3.8	2.5
3. I get poisoned after spraying.	60	33.8	1.3	3.8	1.3
4. I was hospitalized after the spraying.	65	31.3	1.3	.	2.5
Environmental health after spraying					
1. I throw the agricultural drug boxes on the edge of the field.	46.3	22.5	2.5	27.5	1.3
2. I burn the agricultural drug boxes.	3.8	6.3	2.5	60	27.5
3. I clean the spraying tools and equipment in a stream, fountain, or in such a way that such water mixes with the soil.	45	37.5	7.5	7.5	2.5
4. I clean the spraying tools and equipment at the edge of the field with bulk water or in such a way that it mixes with the soil.	41.3	32.5	10	13.8	2.5
5. I know that the spraying I have done until the harvest is a lot, and I feel sad.	43.8	32.5	15	7.5	1.3

Table 22 shows the farmers' opinions about consumer, farmer, and environmental health after pesticide application. The results show that farmers are undecided about the effects of pesticide residues on consumer health, and they need to be informed. It was determined that most of the farmers did not have a problem with their health after spraying, and the number of farmers adversely affected by pesticides was low. When the opinions about environmental health were examined after spraying, it was determined that 60% of the farmers burned empty pesticide packages. It has been determined that a significant part of the farmers clean their spraying tools and equipment without mixing with the natural water sources and the soil near these sources. Similarly, when they use bulk water, they clean it without mixing it with the soil at the edge of the field.

CONCLUSION

The rapid increase in the world population increased the production volume with the industrial revolution. To meet the increase in consumer demand for food products, getting the highest yield per unit area in agricultural areas has been among the producers' goals. The use of pesticides enables diseases and pests to be controlled, thus positively affecting product performance. However, on the other hand, the use of pesticides in industrialization processes causes deterioration in the natural balance of the ecosystem and plays a role in climate change and global warming.

In this study, the profile of the farmer producing grain maize in the Yunak District of Konya rural was examined, and their demographic and general characteristics were determined. Awareness levels of farmers about pesticide use are discussed under the headings of consumer health, farmer health, and environmental problems before, during, and after spraying. Attitudes and behaviors of grain maize producers on pesticide use were determined, and agricultural pesticide supply characteristics in Herbicide, Fungicide, and Insecticide types were determined. It has been determined that the farmers are mostly in the middle age group (49.8 years), and their education level is at the average high school level. It has been determined that the average size of the grain maize fields produced is 174.8 decares, and the average yield is 1487.3375 kg/da. The yield in the study area is above the average yield of Turkey (1,123 kg/ha) and Konya (1,015 kg/ha). Grain maize producers within

the scope of the study stated that they are generally familiar with diseases and pests (insects, grass, fungus) in grain maize. Grain maize producers stated that they determined the pesticide type based on their own experience and the recommendations of the pesticide dealers. It has been determined that grain maize producers generally apply pesticides based on the advice of pesticide dealers. When farmers' attitudes and behaviors are examined before, during, and after spraying, it has been observed that farmers need to be informed about consumer and environmental health. It is thought that training or seminar activities will be beneficial to improve the views of farmers on environmental awareness and consumer health in pesticide applications. In the region where the research was carried out, it was observed that the empty pesticide packages were burned to a large extent, and the cleaning water of the spraying tools and equipment was thrown to the side of the field. The attitudes, as mentioned earlier, of the farmers may cause harm to other living things apart from the agricultural pests that are desired to be controlled, and the deterioration of the ecological balance may result in the ongoing process. It is thought that collecting agricultural wastes under control or establishing agricultural waste storage facilities in areas where agricultural production is possible will contribute positively to the health of the ecological balance. Grain maize producers widely and frequently use pesticides in maize grain production. During the survey, the farmers expressed that they want to use environmentally friendly and less dangerous pesticide than the ones they currently use. Increasing the effectiveness of agricultural consultants in the research region will increase the farmers' knowledge level and will positively contribute to the optimized progress of agricultural practices. It is thought that the correct and optimal use of pesticides will also contribute positively to the economic performance of grain maize production. Opening a properly grown and protected agricultural product to large markets will also provide economic contributions to the farmers. In the literature review, no study has been found examining the use of pesticides in grain maize production and the producers' knowledge levels, attitudes, and behaviors regarding pesticide use. It is thought that the results will be a source of information for the decision-making units and researchers in the agricultural field regarding the region where the study is conducted.

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CHAPTER 10

THE HISTORICAL LOCAL PLUM AND PEACH CULTIVARS IN YUKSEKOVA (HAKKÂRI) ECOLOGICAL CONDITIONS

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1. INTRODUCTION

Turkey has the appearance of a continent with different climatic characteristics. Turkey, which has a great cultivar richness, is almost a natural museum of fruit culture due to its different climatic characteristics and geographical location suitability and fruit species richness. The most of the fruit species and cultivar, especially stone and pome fruit species, have been grown in almost every region of Turkey, which are the homeland of many fruit species, since ancient times, and they have an important value in the country's fruit growing. It is accepted that the homeland of plum is Anatolia, around the Caspian Sea and the Caucasus. Therefore, Anatolia constitutes an important gene source for plum. The number of rich plum varieties has increased with the import of P. domestica, which constitutes the cultivars, and the eastern plum species (Ozbek, 1978). The plum production of the world, half of which is produced by China is 12,608,678 tons. According to 2018 data of FAO, it produced 296,878 tons of plums in Turkey (Anonymous, 2019a). In Hakkari and its districts, 1,083 tons of plums are produced in an area of 1,216 decares. Şemdinli district is in the first place with 339 tons and Yüksekova is in the second place with 314 tons(Anonymous, 2019b).Peach (Prunus persica L.), which is native to East Asia and China, is one of the fruit species that can adapt to different climatic conditions. Peach cultivation in the world is carried out between the latitudes of 25-45˚ in the south and north of the equator (Ozbek, 1978). Peach is a type of fruit that has a very wide growing area in the world. The largest peach and nectarine producer countries in the world are respectively; China (15.217.797 tons), Italy (1.090.678 tons), Spain (903.809 tons), Turkey (789.457 tons) and the USA (700.350 tons). World peach and nectarine production is 24,453,425 tons according to FAO. Considering the production of Turkey, according to the data of the Turkish Statistical Institute (TUIK) in 2019, 685,973 tons of peaches were produced in an area of 379,424 da. Yield per tree in Turkey is 46 kg. According to TUIK 2019 data, the provinces that produce the most peaches in Turkey are Çanakkale (126,847 tons), Mersin (113,795 tons), Bursa (109,916 tons), Izmir (74,200 tons) and Denizli (52,581 tons). According to the data of the Turkish Statistical Institute in 2019, in Hakkari province and its districts, a total of 457 tons of peaches are produced in an area of 127 decares (Anonymous, 2019c).

Turkey, which has very rich genetic resources and is among the homelands of plum and peach, has very suitable ecological conditions for plum and peach cultivation and the commercial cultivars adapted to ecological conditions are grown in almost all regions of the country. However, the local peach and plum cultivars that have survived until today are the important cultural and genetic riches of the country.

These local cultivars, which are especially appreciated for their taste and flavour, offer genetic richness with their morphological and pomological characteristics. So these local peach and plum cultivars should be accepted as a cultural inheritance and transferred to the future. In addition, this genetic richness creates important alternatives in terms of the cultivar breeding suitable for different soil and climatic conditions, and the cultivar developing suitable for different domestic and foreign market demands (Akcay et al., 2009; Bostan and Acar 2009). In Turkey, which has a very large fruit growing culture, the many studies have been carried out to reveal the quality characteristics and their importance by determining the phenological, pomological and technological characteristics of the local cultivars on fruit species such as apple (Ozrenk et al., 2011; Kirkaya et al., 2014; Vurgun and Aslantas, 2015; Coskun and Askin; 2016) and pear (Yarilgac and Yildiz, 2001; Uzunismail, 2010; Karadeniz and Corumlu, 2012; Polat and Bagbozan, 2017; Oturmak et al., 2017). However, there are not many studies about local plum and peach cultivars. In the study, it was aimed to determine the phenological, pomological, morphological and chemical properties of local plum (Huluhoh, Huluzer, Hulusor, Hulures) and peach (Hohosipi) cultivars in Yuksekova (Hakkâri) region.

2. MATERIAL and METHODS

2.1. Material

The trees of the local plum ((Huluhoh, Huluzer, Hulusor, Hulures)) and peach (Hohosipi)) cultivars, which have been growth in the form of scattered and orchards for a long time in Yesiltas (37° 27' 0.402" North, 44° 4' 41.672" East) and Daglica (37° 21' 53.35" North, 44° 3' 37.679" East) villages of Yuksekova district, constitute the material of the study. These local fruit varieties are grown by farmers as a cultural heritage and are produced because they provide economic gain. Local people see these local fruit varieties as organic fruit varieties, so there is a high demand for these fruits, which have

been appreciated by the public. Yuksekova, where the continental climate is dominant, it is in the "hot humid continental (Dsa)" climate class in summers according to Köppen-Geiger' climate classification (Peel et al., 2007). Yesiltas and Daglica villages, where is altitude of 1340-1565 meters, have microclimate climate characteristics with the effect of the mountains and the Avasin river, and therefore the subtropical climate fruit species such as pomegranate, fig, olive and persimmon can be grown in the region. The soil of the region consists of the fertile soils in colluvial structure consisting of the sediments carried by the surface waters, rivers and streams.

Figure.1. The study area and orchards

2.2. Methods

In the study carried out in 2018 and 2019 in order to determine the characteristics of the historical local peach and plum cultivars in Daglica and Yesiltas (Yuksekova) villages, 10 trees were determined for each cultivar, and the morphological, phenological, pomological and chemical characteristics of these trees and their fruit were determined by analysis and measurements as indicated following.

Morphological characteristics: The tree's age (years) was determined by counting the branches back in the trees or according to the statement of the orchard owner. Tree height (cm) was measured at the end of the growing season with a tape measure and the soil surface was considered as 0 (zero). Canopy width (cm) and height (cm) were measured to determine tree canopy volume. For canopy width, two measurements were made in the north-south and east-west directions in the central part of the tree canopy, and the average of these

was taken. The canopy height was determined by measuring the distance between the point where the first branches formed and the top of the canopy with meters. By using these measurements, the canopy volume values were calculated with the formula $V=\pi r^2.h/2$ (Yildirim and Celik, 2003). In the formula, V: volume, π: constant value, r: radius, h: height. The periodicity tendency was evaluated by considering the research results and the opinion of the breeder together. The tree habitus is grouped as upright, semi-upright and splay while the growth vigor of the trees grouped as weak, semi-vigorous and vigorous. The tree trunk circumference (cm) was measured by meters at the beginning and end of the vegetation, 5 cm above the grafting area**.** Shoot length (cm) was measured with meter on ten shoots from ten trees representing each cultivar in the dormant period. The shoot diameter (mm) was measured with a digital caliper (0.01 mm sensitive) on ten shoots from ten trees representing each cultivar in the dormant period. Leaf characteristics (leaf length and leaf width) were measured by digital caliper (sensitive to 0.01 mm) by randomly taking 10 mature leaves from each cultivar.

Phenological characteristics: The bud swelling and breaking were determined by recording the dates when the buds began to swell and burst on the trees. The period when 10% of the flowers opened was accepted as the first blooming, the period when 80% of the flowers opened was considered as full blooming and the period when 80% of the petals of the flowers fell was accepted as the end of the flowering, and the dates were recorded (Orman, 2005). Harvest time was determined by considering the harvest criteria such as fruit colour and fruit size. The number of the days from full blooming to harvest date (FBHDN) was determined by calculating the days in between the full blooming and harvest time on the trees. Leaf yellowing is when 70% of the leaves begin to turn yellow (Tekintas et al., 2006); the leaf drop is taken as the period in which the leaves turn yellow and fall 90%.

Yield: For, yield per trunk cross-sectional area, firstly, the trunk diameter was measured 15 cm above the graft union with a digital caliper with a sensitivity of 0.01 mm. The trunk cross-sectional area (cm^2) was calculated by using the formula $TCSA= \pi.r^2$. Then, yield per tree (kg/tree) was determined

by weighing the all fruit of the tree. The yield per trunk cross-sectional area was determined by dividing the yield per tree by the trunk cross-sectional area.

Pomological characteristics: The following measurements and analyzes were made by taking 20 fruit from the trees representing each cultivar. The fruit weight was measured with a digital scale (Radwag, Poland) with a sensitivity of 0.01 g and the fruit weight was determined by taking the average and expressed as g. For the fruit firmness, the fruit peel was cut at three different locations on the equatorial region of the fruit and measured as kg with the 11.1 mm tip of the penetrometer (Effegi brand, model FT-327; MoCormick Fruit Tech, Yakima, WA). Fruit length (mm) was found by measuring the longest distance between the fruit's stem pit and flower pit, and the fruit width (mm) was found by measuring the widest part of the fruit in the equatorial region with a digital caliper sensitive to 0.01 mm (Kaya, 2008). The fruit shape index, which is obtained by dividing the fruit length (mm) by the fruit diameter (mm), is accepted as flat if it is between 0.81-0.92, round if it is between 0.93-1.04, and as long as 1.05 and above (Guleryuz and Ulkumen, 1972). The kernel's number (piecs/fruit) was determined by counting the fruit's kernel. The sensory observations, taste, aroma and juiciness of fruits were determined by sensory observations; classified as moderate, good, very good.

Chemical characteristics*:* The fruit was shredded with a blender and made homogeneous, and the obtained homogenate was passed through a cheesecloth and fruit juice was obtained. Sufficient amount of juice was dropped into a digital refractometer (PAL-1, McCormick Fruit Tech., Yakima, Wash.) for the measurement of soluble solids content (SSC) and the value on the screen was recorded as %. pH was measured with a pH meter (Hanna, model HI9321). For titratable acidity (TA) measurements, 10 ml of the obtained juice was taken and 10 ml of distilled water was added on it. Then, samples were expressed in terms of malic acid (g malic acid 100 ml-1) based on the amount of NaOH spent in titration with 0.1 N sodium hydroxide until pH 8.1 was reached.

3. RESULTS and DISCUSSION

3.1. Phenological properties

In the phenological observations made from local plum cultivars, bud growth occurred between April 4-10 (Huluzer) at the earliest and April 16-24 (Hulures) at the latest in 2018 and between April 7-15 (Huluzer) and April 14-20 (Hulures) in 2019 bud burst took place between April 9-15 (Huluzer) and April 22-29 (Hulures) in 2018, and between April 12-19 (Huluzer) and April 19-25 (Hulures) in 2019 (Tables 1, 2, 3 and 4). It was determined that the beginning of flowering in cultivars was between 20-28 April (Huluzer) and 10-17 May (Hulures) in 2018, 23-30 April (Huluzer) and 7-14 May (Hulures) in 2019, full flowering occured between 1-15 May and May 20-26 (Hulures) in 2018, and between May 6-12 (Huluzer) and May 19-23 (Hulures) in 2019. On the other hand, it was determined that the end of flowering in the examined varieties was between 18-24 May (Huluzer) and 25-30 May (Hulures) in 2018, and between 16-22 May (Huluzer) and 23-29 May (Hulures) in 2019 (Tables 1, 2, 3 and 4). In local plums, there is a 2-5 day difference in the dates of bud swelling, bud breaking, beginning of flowering, full flowering and end of flowering in varieties in 2019 compared to 2018. In 2018 and 2019, the earliest bud swelling occurred in Huluzer, and the latest in Hulures cultivar. The earliest bud bursting was in Huluzer while the latest was in Hulures cultivar. The earliest flowering cultivar in both years was Huluzer. In 2018 and 2019, flowering has started at the latest in Hulures and Hulusor cultivars. Although the flowering period varies according to the cultivar, it lasted 11-17 days in 2018 and 9-12 days in 2019 with the effect of ecological factors (Tables 1, 2, 3 and 4). In similar studies conducted in different ecologies of Turkey, it has been determined that there are significant changes in the flowering dates of plums. Beyhan (2005), in his study in Darende District of Malatya province, determined 13 local plum cultivar that are widely grown in the region. The bud bursting and flowering of the examined cultivars took place within the 1st and 3rd weeks of April with a maximum difference of 10 days, depending on the cultivars. Cocen et al. (2018) reported that the earliest flowering cultivar was President (8-12 April), and the latest flowering cultivar was Stanley (11-15 April), the flowering period varied between 12-14 days, and leaf fall was on November 15-17. It can be said that ecological factors, altitude, cultivar and cultural practices are effective in the emergence of different results in the

studies. In the phenological observations of local plums, the harvest time was between 1-8 September (Huluzer) and 22-30 September (Hulures) in 2018, and between 27 August-5 September (Huluzer) and 5-14 October (Hulureş) in 2019. The leaf yellowing occurred between October 12-25 (Huluzer) and November 5-20 (Hulures) in 2018, and between October 10-20 (Huluzer) and November 3-15 (Hulures) in 2019. It has been determined that the leaf fall in cultivars took place between 5-12 November (Huluzer) and 2-10 December (Hulures) in 2018, and between 10-15 November (Huluzer) and 5-14 December (Hulures) in 2019 (Tables 1, 2, 3 and 4). In similar studies conducted in different ecologies of Anatolia, it was determined that the harvest times of plums were between 15 June and 13 September (Bilgu and Seferoglu, 2005); 1 August-18 September (Karamursel et al., 2007), mid-June-first week of September, 24 July-16 September (Subası, 2013), 11 July-13 September (Acar, 2016), 1 August-10 September (Celik and Gulcan, 2018) and 18 August- 14 September (Cocen et al. (2018). In this context, it is thought that the differences in phenological characteristics are caused by the changes in the cultivar and environmental conditions. In the phenological observations made in Hohosipi, a local peach cultivar, bud swelling was 8-13 April in 2018, 5-16 April in 2019, bud breaking was 15-19 April in 2018, and 18-23 April in 2019. It was determined that the beginning of flowering took place on 20-27 April in 2018, 25-28 April in 2019, full flowering on 5-12 May in 2018 and 7-13 May in 2019. The end of the flowering was determined as 15-20 May in 2018 and 20-25 May in 2019 (Table 5). The flowering period from local peaches lasted an average of 16 days in 2018 and 13-16 days in 2019. It is seen that there is not much difference between the phenological characteristics over the years. In similar studies conducted in different ecologies of Turkey, it has been determined that there are changes in the flowering dates of peach. In the study carried out by Gur and Pirlak (2011) at Eğirdir Fruit Research Institute, the dates of the bud swelling and the beginning of flowering were determined as March 8 and March 29 respectively. Bolat and Ikinci, (2016) reported that bud swelling was between 28 February and 13 March, bud breaking between 9 and 24 March, and full flowering between 18 March and 3 April. Harvest date was determined as 10-24 September in 2018, 7-18 September in 2019, leaf yellowing 15-24 October in 2018, 10-20 October in 2019, the leaf fall 4-15 November in 2018, 1-12 November in 2019. The number of days from flowering to harvest was

determined as 128-135 days in 2018 and 123-128 in 2019 (Table 5). Bolat ve Ikinci (2016) reported that pomegranate harvest was done between June 2 (Maycrest) and August 24 (Monroe) and fruit development period in peach cultivars ranged from 74 days (Maycrest) to 151 days (Monroe). In this context, it is thought that the differences in phenological characteristics are caused by the changes in the cultivar and environmental conditions.

3.2. Morphological characteristics

In the measurements made on local plums in Yuksekova (Hakkari) ecological conditions, the lowest tree height in 2018 was 495.62±3.47 cm (Huluhoh), the highest tree height was 875.5±4.11 cm (Huluzer); In 2019, the lowest tree height was 504.50±3.67 cm (Huluhoh), and the highest tree height was 882.20±4.50 cm (Huluzer). In 2018, the widest canopy was 742±3.54 cm (Huluzer), and the narrowest canopy was 412±2.88 cm (Huluhoh); In 2019, the widest canopy was 768.50±3.78 cm (Huluzer), and the narrowest canopy was 440±3.05 cm (Huluhoh) (Tables 1, 2, 3 and 4). Previous studies have reported that the canopy height of plum varies between 1.50 and 5.98 m, and the canopy width varies between 1.00 and 7.40 m (Beyhan, 2005; Karamürsel et al., 2007; Subası, 2013; Celik and Gulcan , 2018; Cocen et al., 2018). When the results of similar studies were compared, it was seen that there were significant differences, and these differences may be due to differences in cultural practices and ecological characteristics. The yield per trunk cross-sectional area of the local plum cultivars was between 0.05 kg/cm² (Hulusor) to 0.09 kg/cm² (Hulures) in 2018, and was 0.07 kg/cm² (Hulusor) to 0.10 kg/cm2 in 2019. cm² (Hulures). The yield per tree was between 29.65 kg (Hulusor) to 46.5 kg (Hulures) in 2018, and was 31.85 kg (Hulusor) to 47.65 kg (Huluzer) (Tables 1, 2, 3 and 4). Bilgu and Seferoglu (2005) reported that the yield per trunk cross-sectional area of plum varied between 0.35 and 0.96 kg while Cocen et al (2018) determined that the yield per tree was 33 kg and the yield per trunk cross-sectional area was 0.06 - 0.22 kg/cm². Again, Subası (2013) determined that the yield per tree was 12.62-25.37 kg, and the yield per trunk cross-sectional area was 0.027-0.117 kg/cm² in the study he conducted in Kahramanmaraş. It is seen that the yield values obtained from our study are compatible with the results of other researchers.

In the ecological conditions of Yuksekova (Hakkari), the shoot length of local plum cultivars was 24.5 cm (Huluhoh), to 41.6 cm (Hülüzer) in 2018, , and it was 26.71 cm (Huluhoh), to 44.2 cm (Huluzer); The shoot diameter was 5.92 mm (Huluhoh), 6.84 mm (Huluzer) in 2018, and was 6.13 (Huluhoh), 6.90 mm (Huluzer) in 2019. In both years of the study, it was determined that the cultivar with the strongest shoots in terms of shoot length and shoot thickness was Huluzer, and the cultivar with the weakest shoots was Huluhoh (Tables 1, 2, 3 and 4). In morphological measurements of Hohasipi local peach cultivar in Yuksekova (Hakkari) ecological conditions in 2018 and 2019, it was determined that the tree height was 490 and 550 cm, and canopy width was 585 cm and 624.45, respectively (Table 5). The yield per tree and trunk cross-sectional area was 33.6 kg and 0.470 kg/cm² in 2018, it was determined as 37.50 kg and 0.521 kg/cm² in 2019 respectively (Table 5). Topak (2014) determined that the yield per trunk cross-sectional area of peach was between 0.542 and 0.584 kg/cm² and the yield per tree is between 33.77 and 37.29 kg. Bolat and Ikinci suggested that the yield per tree in peach is between 22.97 and 39.19 kg. It is seen that the yield values obtained from our study are compatible with the results of other researchers. In the study, the shoot length and shoot diameter values did not change depending on the year, while the average shoot length was 48.9 and the shoot diameter was 6.67 mm (Table 5). However, Topak (2014) reported that the shoot length of the peach was between 76.6 and 85.5.

3.3. Pomological characteristics

In both years of the study, in local plum cultivars, the largest fruit were obtained in Hulures, while the smallest fruit were harvested in Huluzer (Tables 1, 2, 3 and 4). In accordance with the study, Celik and Gulcan (2018) determined that the fruit weight of plum was 8.66 to 25.59 g, and Beyhan (2005) reported that it was between 12.63 g to 29.17 g. Again, Koyuncu et al. (1993) determined that the fruit weight of local plum cultivars was between 8.30 g and 29.50 g. However, in the different studies (Onal ve Cinsoy, 2003; Subası, 2013), it has been suggested that the fruit weight of plum is relatively higher and varied between 19.0 g and 83.70 g. It is thought that factors such as ecological conditions, cultivar, altitude and cultural practices are effective in obtaining different results in studies. In both years, the highest values in terms of fruit firmness (6.42 lb) were recorded with Hulures cultivar while the fruit

of Huluzer cultivar (1.28 lb) were found to be softer (Tables 1, 2, 3 and 4). In his study, Subası (2013) reported that the fruit flesh firmness values in plums ranged from 3.51 lb to 9.11 lb. It can be said that the findings of our study are in accordance with the results of other researchers.

In Hohasipi local peach cultivar, in 2018 and 2019 respectively, the fruit weight was 82.14 g; and 80.52 g; fruit length 48.78 mm and 47.50 mm; fruit width was determined as 45.02 mm and 44.16 mm (Table 5). Kaska et al. (1992) reported that the fruit weight of peach was between 101.2 g and 209.0 g, and Bolat and Ikinci, (2016) reported that it was between 78.19 g and 218.73 g. Compared to the results of the study, the fruit obtained in our study were relatively small, and this was due to the fact that the cultivar in the study was a local cultivar, and the ecological factors were different and the cultural practices were not done properly. Fruit firmness of Hohasipi local peach cultivar was determined as 3.02 lb in 2018 and was 3.19 lb in 2019 (Table 5). Gur and Pirlak (2011) determined that the fruit firmnes in peach was between 0.96 kg/cm^2 and 2.90 kg/cm^2 while Bolat and Ikinci (2016) reported that the fruit firmness values varied between 2.59 and 3.72 kg/cm2.

3.4. Biochemical characteristics

The ratio of SSC of local plum cultivars was found between 11.82% (Huluzer) and 20.03% (Hulusor) in 2018, and was between 11.57% (Huluzer) and 21.05% (Hulusor) in 2019 (Tables 1, 2, 3 and 4). In similar studies, it was reported that the ratio of SSC of plum was between 17.60% to 19.30%; (Cocen et al., 2018), 10.8% to 17.0% (Sen et al., 1992) and 12.28% and 25.55% (Onal and Cinsoy, 2003). The pH and titratable acidity values in plum cultivars varied between 3.10 and 3.80 and 0.57% and 1.47%, respectively. In both years, the highest pH ratio was measured in Hulures and the lowest in Hulusor cultivars while the opposite was true for acidity. The highest acidity values were recorded with Hulusor variety, but the lowest values were measured in Hulures variety (Tables 1, 2, 3 and 4). Consistent with our study results, Cocen et al. (2018) reported that the pH value in plums varied between 3.2 and 3.71 and the titratable acidity ratio varied between 0.64% and 0.86%. Whereas, Balik (2005) determined a lower titratable acidity rate (0.08% to 0.20%) in plum. It can be said that fruit maturity levels are effective in the emergence of different results.

In Hohasipi local peach cultivar, in 2018 and 2019 respectively, SSC oranı %13.46 ve %14.11; titratable acidity %0.46 ile %0.48 olarak belirlendi. In Hohasipi local peach cultivar, in 2018 and 2019 respectively, SSC ratio was 13.46% and 14.11%; titratable acidity was determined as 0.46% to 0.48% (Table 5). Gur and Pirlak, (2011) reported that the SSC rate in peach was 10.7% to 16.6%, Ozekici (2005) reported that the acidity value was between 0.1% and 0.3%. SSC content in fruit can be affected by factors such as ecological characteristics, harvest time and variety. It can be said that these factors are effective in the differences in the results of the studies.

3.5. Fruit and shoot growth

As a result of measurements made at 14-day intervals in order to monitor fruit development in local plums, fruit width and fruit length in plums were examined in three stages according to their daily growth rates. It was determined that fruit development was fast in the first and last stages of the examined cultivars and slow in the middle stage. In the experiment, the growth of the fruit showed differences dependent on the cultivars. The first rapid growth phase, which is the first stage of fruit growth in plums, lasted 36 days for Huluhoh, 47 days for Huluzer, 33 days for Hulures, and 33 days for Hulusor. The slow growth phase lasted days in all cultivars. The second rapid growth phase lasted 56 days in Huluhoh cultivar, 42 days in Huluzer cultivar, and 70 days in Hulures and Hulusor. In all cultivars, with the continuous increase in fruit length and fruit width during the growing period, it was observed that fruit length developed faster than fruit width (Hulusor and Hulures) and fruit length developed at the same rate in fruit width (Huluhoh and Huluzer). When the fruit development stages were examined, it was determined that the fruit of all the studied cultivars grew by forming a double sigmoid curve. In order to monitor shoot development, shoot length and shoot diameter were measured at 14-day intervals in local plums. There were differences between the cultivars in the shoot development stages. In the shoots of all cultivars, it was determined that the shoot development was fast in the first stage, but the shoot development became stagnant in the last stage and the rate of development slowed down almost to nothing. When the shoot lengths were examined, the cultivars completed their the first phase of rapid development in 7 June-5 July, After this stage, the shoot length gradually slowed down (Table 1, Table 2, Table 3, Table

4). Subasi (2013), who stated that the shoot length of the plum changes depending on the cultivar, determined that stronger shoots occurred in the Formosa cultivar. When the data obtained in the studies are compared with the standard cultivars, there are partial differences. The reasons for this are thought to be due to factors such as cultivar difference, pruning methods, soil structure, ecological balance. As a result of the measurements made at 14-day intervals in order to monitor the fruit development of the local peach cultivar (Hohasipi), the fruit width and fruit length of the local peach were discussed in three stages according to the daily growth rates. It was determined that fruit development was fast in the first and last stages and slow in the middle stage (Table 5). The first rapid growth phase in fruit lasted 41 days, the slow growth phase lasted 28 days and the second rapid growth phase lasted 56 days. It was observed that fruit length developed faster than fruit width during the growth period. When the fruit development stages were examined, it was determined that the fruits grew by forming a double sigmoid curve. In order to monitor shoot growth, shoot length and shoot diameter were measured at 14-day intervals. It was determined that while shoot growth was fast in the first stage, but slowed down in the last stage. When the shoot lengths are examined, 59.61% of shoot growth was completed in the 28-day period, where rapid development was experienced on 7 June-5 July. After this stage, the shoot growth gradually slowed down (Table 5). Topak (2014) suggested that the shoot length varied between 76.66 cm and 85.55 cm in peach.

4. CONCLUSSION

In the study, it has been seen that the local plum and local peach fruit cultivars grown in Yüksekova (Hakkari) district and the surrounding villages (Yesiltas, Daglıca) have suitable ecology in terms of cultivation. There are local cultivars with characteristics that can be standard cultivars. The people of the region have made a significant contribution to the economy of the region, especially the family, by cultivating these local fruit species for many years. Local cultivars that are widely grown in the region can be brought to the potential to meet the needs of the region by being grown in a more controlled and more conscious way in rural areas. The presence of local cultivar richness in the region will make significant contributions to the development of the local economy in the future. This study is important to identify the local plum and

peach cultivar of the region and to make them known. Among these local cultivars, the ones that can be standard cultivars should be selected, and the necessary technical information should be given to the growers about cultivation, and new orchards should be established for the cultivation of more and higher quality fruit. Thus, it is clear that the local cultivars with good characteristics, which come to the forefront as a result of our research and adapt to the local ecology, will be very effective in increasing the fruit production potential of the region. It provides an advantage in terms of not being damaged by late spring frosts due to the fact that there are areas with microclimate climate in the region, the vegetation period is late and the flowering is late in local varieties. On the other hand, since the early frosts of autumn do not occur during the harvest period in the region, no problems such as chilling or freezing are expected in the fruits. Therefore, the transition to standard cultivation with local cultivas for the region will be seen as an economic advantage. Among the local plum cultivars, Hulures, Huluhoh and Hulusor were found promising. The most ideal cultivar in terms of pomological characteristics is Hulures. It is consumed as a table and has a long storage life. Huluhoh is a delicious local plum cultivar with a peach flavor, has a good storage life, is resistant to diseases, and is liked by the local people. Hulusor is a quality type in terms of fruit weight and fruit size when looking at the local plum variety, it is consumed as table and dried. Since the fruit structure of Huluzer is small and its taste is sour, it is mostly consumed in drying. Although it has lower values compared to standard cultivars in terms of pomological, phenological and morphological, it can make a difference in terms of taste and productivity. Under the ecological conditions of Yuksekova (Hakkari), it is no doubt that the expansion of the cultivation of the varieties we have determined in the region and the determination of different cultivars specific to the region by making more extensive adaptation trials will make a significant contribution to the region's fruit growing and economy.

Table 1. The characteristics of "Hulusor" local plum cultivar.

Morphological characteristics	
Tree age (year)	14-15
Tree height (cm)	717.40
Canopy volume (m^3)	18.93
Canopy shape	Upright
Tree growth vigor	Semi-vigorous
Shoot length (cm)	30.85
Shoot diameter (mm)	6.21
Leaf length (mm)	67.13
Leaf width (mm)	35.66
Phenological characteristics	
Bud swelling	7-17 April
Bud breaking	15-25 April
First blooming	1-12 May
Full blooming	15-22 May
The end of the flowering	23-30 May
Harvest date	17-30 September
FBHDN* (day)	125-131
Leaf drop	11-20 November
Yield	
YPTCSA** (kg/cm^2)	0.05
Yield per tree (kg/tree)	28.86
Pomological characteristics	
Fruit weight (g)	24.53
Fruit length (mm)	41.43
Fruit width (mm)	32.47
Fruit shape index	Long
Fruit firmness (lb)	2.54
Chemical and sensory characteristics	
Soluble solids content (%)	19.97
Titratable acidity (%)	1.43
pH	3.12
Fruit juiciness	Good
Fruit taste	Tart
Fruit aroma	Good

*FBHDN: Number of days from full blooming to harvest date. ** YPTCSA: Yield per trunk cross-sectional area

Table 2. The characteristics of "Hulures" local plum cultivar.

Morphological characteristics	
Tree age (year)	12
Tree height (cm)	667.9
Canopy volume (m^3)	18.24
Canopy shape	Upright
Tree growth vigor	Vigorous
Shoot length (cm)	33.72
Shoot diameter (mm)	6.50
Leaf length (mm)	68.90
Leaf width (mm)	37.85
Phenological characteristics	
Bud swelling	14-24 April
Bud breaking	19-29 April
First blooming	7-17 May
Full blooming	19-26 May
The end of the flowering	23-30 May
Harvest date	5-20 October
FBHDN* (day)	139-147
Leaf drop	2-14 December
Yield	
YPTCSA** (kg/cm^2)	0.08
Yield per tree (kg/tree)	45.55
Pomological characteristics	
Fruit weight (g)	51.81
Fruit length (mm)	47.82
Fruit width (mm)	38.90
Fruit shape index	long
Fruit firmness (lb)	6.35
Chemical and sensory characteristics	
Soluble solids content (%)	18.87
Titratable acidity (%)	0.58
pH	3.77
Fruit juiciness	Good
Fruit taste	Sweet
Fruit aroma	Good

*FBHDN: Number of days from full blooming to harvest date. ** YPTCSA: Yield per trunk cross-sectional area

Table 3. The characteristics of "Huluzer" local plum cultivar.

Morphological characteristics	
Tree age (year)	14-15
Tree height (cm)	930.4
Canopy volume (m^3)	79.35
Canopy shape	Semi-upright
Tree growth vigor	Semi-vigorous
Shoot length (cm)	39.18
Shoot diameter (mm)	6.55
Leaf length (mm)	38.10
Leaf width (mm)	22.30
Phenological characteristics	
Bud swelling	4-15 April
Bud breaking	9-19 April
First blooming	20-30 April
Full blooming	1-15 May
The end of the flowering	16-24 May
Harvest date	27 Agust-8 Sep.
FBHDN* (day)	113-123
Leaf drop	5-15 November
Yield	
YPTCSA** (kg/cm^2)	0.07
Yield per tree (kg/tree)	41.40
Pomological characteristics	
Fruit weight (g)	8.21
Fruit length (mm)	21.46
Fruit width (mm)	24.54
Fruit shape index	Round
Fruit firmness (lb)	0.97
Chemical and sensory characteristics	
Soluble solids content (%)	11.77
Titratable acidity (%)	0.64
pH	3.12
Fruit juiciness	Very good
Fruit taste	Sour
Fruit aroma	Good

*FBHDN: Number of days from full blooming to harvest date. ** YPTCSA: Yield per trunk cross-sectional area

Table 4. The characteristics of "Huluhoh" local plum cultivar.

Morphological characteristics	
Tree age (year)	14-15
Tree height (cm)	502.60
Canopy volume (m^3)	10.80
Canopy shape	Upright
Tree growth vigor	Semi-vigorous
Shoot length (cm)	24.32
Shoot diameter (mm)	5.80
Leaf length (mm)	70.02
Leaf width (mm)	36.35
Phenological characteristics	
Bud swelling	5-16 April
Bud breaking	12-20 April
First blooming	25 April-1May
Full blooming	5-7 May
The end of the flowering	18-28 May
Harvest date	7-20 September
FBHDN* (day)	120-128
Leaf drop	7-20 November
Yield	
YPTCSA** (kg/cm^2)	0.07
Yield per tree (kg/tree)	33.25
Pomological characteristics	
Fruit weight (g)	29.40
Fruit length (mm)	35.62
Fruit width (mm)	35.05
Fruit shape index	Round
Fruit firmness	3.78
Chemical and sensory characteristics	
Soluble solids content (%)	17.27
Titratable acidity (%)	0.75
pH	3.35
Fruit juiciness	Very good
Fruit taste	Sweet
Fruit aroma	Good

*FBHDN: Number of days from full blooming to harvest date. ** YPTCSA: Yield per trunk cross-sectional area

Table 5. The characteristics of "Hohosipi" local peach cultivar.

Morphological characteristics	
Tree age (year)	14
Tree height (cm)	490.14
Canopy volume (m^3)	22.32
Canopy shape	Splay
Tree growth vigor	Weak
Shoot length (cm)	48.24
Shoot diameter (mm)	6.58
Leaf length (mm)	76.24
Leaf width (mm)	31.08
Phenological characteristics	
Bud swelling	8-13 April
Bud breaking	15-19 April
First blooming	20-27 April
Full blooming	5-12 May
The end of the flowering	15-20 May
Harvest date	10-24 September
FBHDN* (day)	128-135
Leaf drop	4-15 November
Yield	
YPTCSA** (kg/cm^2)	0.09
Yield per tree (kg/tree)	43.60
Pomological characteristics	
Fruit weight (g)	82.14
Fruit length (mm)	48.78
Fruit width (mm)	45.02
Fruit shape index	Long
Fruit firmness (lb)	3.02
Chemical and sensory characteristics	
Soluble solids content (%)	13.46
Titratable acidity (%)	0.46
pH	3.85
Fruit juiciness	Good
Fruit taste	Sweet
Fruit aroma	Good

*FBHDN: Number of days from full blooming to harvest date. ** YPTCSA: Yield per trunk cross-sectional area

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CHAPTER 11

FRUIT GROWING IN KIRŞEHIR PROVINCE

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1. INTRODUCTION

Kırşehir ranks 68th among 81 provinces with a population of 223 025 and is the least populated province of the TR71 region (Anonymous, 2015). Kırşehir province, located in the Central Kızılırmak section of the Central Anatolia Region, is between 33º 30'-34º 50' east longitudes and 38º 50'-39º 50' north latitudes. Its surface area is 6 665 km^2, and it constitutes 0.8% of Turkey's surface area and 2.9% of the Central Anatolia region's lands. Kırşehir ranks 53rd among 81 provinces in terms of surface area. It is surrounded by Nevşehir in the east and southeast, Niğde and Aksaray in the south, Ankara in the west and southwest, Kırıkkale in the northwest, Yozgat in the north and northeast. The altitude of the city center is 985 meters from the sea, and the average of its land is 64.5% plateau, 17.2% mountainous area and 18.3% plain. In Kırşehir, vegetation with steppe appearance is dominant. The reason why the region, which had forests in ancient times, is poor in vegetation is the negative human effects and the terrestrial effect of the climate for centuries. Kırşehir, located in the center of the Central Anatolia region, is among the easily accessible provinces of Turkey due to its highway transportation from east to west and from north to south. Provincial lands generally consist of 900-1200 m high plateaus. Malya Plain, on which Malya Agricultural Enterprise, which is popularly called desert, was founded, is the largest plain of the province with an area of 400 km^2. Provincial lands are located in the Kızılırmak basin. There are many small and large rivers in the province and these rivers flow into Kızılırmak and Delice Rivers. There are hardly any natural lakes in the province, and Seyfe Lake in Mucur district is the only natural lake (Anonymous, 2015). The elevation of this lake above sea level is 1110 meters. In the period when the water is at its highest, the lake area is 15 km^2. The water of the lake is salty and its depth is at most 165 cm.

When the climate of Kırşehir province is evaluated; It has a typical continental climate with cold and rainy winters and hot and dry summers, with rain falling mostly in autumn and spring. Since the Taurus Mountains surrounding Central Anatolia and the North Anatolian Mountain Range do not allow the temperate climate of the Mediterranean and the Black Sea to enter the interior, the features of the continental climate are observed in the region. The average wind speed in Kırşehir is 2.3 m/s. The prevailing wind directions in the province are Northwest, North and Northeast. It blows from the Southeast in

the fastest winds. The average temperature is 11.5ºC, and the average values of long-year meteorological data by months are given in Table 1. The relative humidity is 63%. The annual precipitation average in Kırşehir varies between 350-500 mm. Maximum precipitation occurs in spring and minimum precipitation occurs in summer. The annual precipitation average is 383.2 mm. The distribution of precipitation in the province is uneven and it receives the least amount of precipitation in the summer months when it is needed the most. It is observed that the average temperature values, which are at negative values in January and February, increase by 23.1°C in the summer months. In extreme years, the lowest temperature was recorded as -28ºC in January, while the highest temperature was recorded as 40.5ºC in August (Anonymous 2022a). According to this, it is seen that the average temperature values of the last 10 years are higher in all months compared to the long-term averages, and this difference reaches up to 1.69°C, especially in summer. The annual average temperature value of the last 10 years is 0.67°C higher than the long years. If we look at the annual temperature averages and the trend of change between 1930-2021, the trend of change in annual temperature averages tends to increase. This situation is seen as a negative situation for global warming (Boyacı and Küçükönder, 2021).

Table 1. Average values of long-year meteorological data of Kırşehir province by month (Anonymous, 2022a)

Months	**Observation (year)**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**Average**
Aver. temper.(ºC)	91	-0.2	1.4	5.3	10.7	15.5	19.7	23.1	23.0	18.6	12.7	6.6	2.1	11.5
Mak. temper. (ºC)	91	19.0	20.6	28.0	30.9	34.5	36.2	40.2	40.5	39.0	33.6	26.2	19.9	40.5
Min. temper. (ºC)	91	-28.0	-25.3	-.21.8	-8.2	-1.4	2.6	5.1	5.0	-1.2	-6.6	-21.5	-24.3	-28.0
Humidity (%)	91	78.2	74.3	67.6	62.4	60.2	54.2	47.6	47.6	52.0	61.8	71.9	78.7	63.0
Sun. time (hours)	81	3.2	4.3	5.3	6.8	8.8	10.8	12.0	11.5	9.5	7.1	5.2	3.2	7.3
Monthly total rainfall aver.(mm=kg÷m²)	91	48.3	35.2	39.6	41.0	44.3	36.4	8.3	7.9	13.0	26.2	36.3	48.5	383.2
Av. wind speed (m/sn)	92	1.8	2.1	2.3	2.3	2.1	2.5	3.3	3.1	2.5	1.9	1.7	1.7	2.3
Wind direction (°)	91	N	N	N	N	N	N	N	N	N	N	N	N	N

When we look at the characteristics of the soils of the province, it is seen that the soil is sandy-loam in general, the pH is medium and high, the lime content is high, and the organic matter content is low. When soils are evaluated in terms of nutrients, they are poor in nitrogen, low in phosphorus and high in potassium (Munsuz et al., 1996). Abacı Bayar and Boyacı (2021), in the study they carried out to determine the nutritional status of some orchards in Kırşehir, found that the soils were sandy loam, there was no salinity problem in general, the pH ranged between 8.07 - 8.44, the amount of organic matter in 93.3% of the orchards. Reported that low and high lime in 80% of them.

In this research, 91 years (1930-2021) climatic values obtained from the relevant meteorological stations for Kırşehir were used. The current state of fruit growing in the province and data on production have been compiled from the sources of TURKSTAT (Turkish Statistical Institute), General Directorate of Meteorology (MGM) and Kırşehir Provincial Directorate of Food, Agriculture and Livestock.

2.GENERAL SITUATION OF PROVINCIAL FRUIT GROWING

In terms of agricultural production value, Kırşehir's share in the country remains below 1%. The agricultural production potential of the province; Vegetable production constitutes 53.2%, live animals 46.6% and animal products 10.2%. The fact that only 9.3% of the agricultural areas of the province can be irrigated limits plant production. However, Kırşehir has a total of 4 547 200 decares of agricultural land, of which 421 760 decares can be irrigated. Although the province has a total water potential of 3 296 hm^3/year, 3 221 hm^3/year above ground and 75 hm^3/year underground, only 9.3% of the total agricultural land can be irrigated. On the other hand, 316 420 decares of irrigated land is done by the state, and 105 340 decares are public irrigation. When the soil and topographic situation of Kırşehir is examined, it has been determined that 3 662 220 decares of the 4 547 200 decares of agricultural land are irrigable. Although 80.5 % of the province's agricultural lands are irrigable, only 9.3 % can be irrigated. Kırşehir province is rich in terms of irrigable land but very poor in terms of irrigated land. Kırşehir, which is on the Kızılırmak basin, which is the longest river of our country, cannot benefit from this situation sufficiently (Kıymaz, 2001). Field crops are grown in 66.2% of the agricultural areas of the province, vegetables in 0.6% and fruit in 0.9%. Due to

the irrigation of a small portion of the province's agricultural lands (9.3%) and the low rainfall, dry farming is generally practiced and 32.4 % of the lands are left fallow.

Table 2. Fruit production data of Kırşehir province (Anonymous, 2022b)

Species	Türkiye production amount (tons)	Provincial production amount (tons)	Share in Türkiye (%)
Grape	3 670 000	4 885	0.13
Walnut	325 000	4 672	1.44
Apple	4 493 264	3 193	0.07
Pear	530 349	683	0.13

As a fruit growing activity in the province, species such as grapes, walnuts, apples and pears are cultivated (Boyacı ve ark., 2017). As can be seen from Table 2, the most produced species in the province is grape with 4 885 tons, and the least production is pear with 683 tons. When the share of the species in Türkiye is evaluated, it is seen that walnut is higher than the other species with 1.44%.

Table 3. Production pattern of fruit species grown in Kırşehir (Anonymous, 2022b).

Species	Amount of produc. (tons)	Area (dec.)	Aver. yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	4 672	26 143	16	288 617	133 820	422 437
Apple	3 193	1 647	34	86 090	27 294	113 384
Grape	4 885	10 363	558	-	-	-
Apricot	375	197	15	23 570	3 690	27 260
Almond	123	817	9	14 121	12 731	26 852
Pear	683	49	50	13 658	3 295	16 953
Cherry	198	168	29	6 908	2 525	9 433
Plum	127	12	19	6 822	418	7 240

In terms of production amount in Kırşehir, grapes are in the 1st place with 4 885 tons, walnuts are in the 2nd place with 4 672 tons, and apples are in the 3rd place with 3 193 tons. In terms of area, it is seen that walnut has the

highest production area with 26 143 decares. The increase in the walnut orchard established in the province in recent years is remarkable.

2.1. Situation of central district fruit growing

The area and production values of the fruit species produced in the central district are given in Table 4.

Table 4. Production pattern of fruit species grown in the central district (Anonymous, 2022b).

Species	Amount of production (tons)	Area (decare)	Average yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	608	14 000	20	30 390	33 570	63 960
Apple	779	515	27	27 871	7 949	35 820
Grape	973	1 678	527	-	-	-
Apricot	195	35	13	15 000	275	15 275
Almond	35	119	13	2 651	1 115	3 766
Pear	126	8	30	4 190	595	4 785
Cherry	34	34	20	1 698	165	1 863
Plum	78	7	15	5 197	138	5 335

Among the Kırşehir districts, the district with the highest agricultural production value is the central district. Of the 1 156 120 decares of agricultural land owned by the central district, 52.8% of field crops, 0.7% of vegetables and 0.5 % of fruit are produced. 46% of agricultural lands are left fallow due to limited irrigation opportunities and low rainfall. 172 040 decares of land, which is equivalent to 15% of the district's agricultural lands, can be irrigated. However, it is not possible to say that irrigated agricultural lands are used very effectively. Industrial crops with high added value, forage crops, vegetables and fruits are grown on only 80 000 decares of irrigated agricultural lands. Grape is the most common type of fruit produced in the central district. In the district, mainly table grapes, wine grapes and dried grapes are produced. While apple is in the 2nd place with 779 tons, walnut is in the 3rd place with 6 078 tons. Again in the central district, as in the province, newly established walnut orchards attract attention.

2.2. Status of Akçakent district fruit growing

Area and production values of fruit species produced in Akçakent district are given in Table 5.

Table 5. Production pattern of fruit species grown in Akçakent district (Anonymous, 2022b).

Species	Amount of production (tons)	Area (dec.)	Average yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	12	650	15	810	2 460	3 270
Apple	16	17	39	410	500	910
Grape	67	712	212	-	-	-
Apricot	3	2	15	200	200	400
Pear	4	10	24	170	40	210
Cherry	2	14	17	120	140	260

The district is at an altitude of 1410 m and is located in the most mountainous part of the province. 48% of its land is forest and it is the district with the largest forest area in Kırşehir. 60 500 decares of land, which is equivalent to 24.3% of the total agricultural land (249 140 decares), can be irrigated. Field crops are grown in 72.9% of the 131 134 decares of agricultural area used and fruit is grown in 1 %. Vegetable production is negligible. 26.1% of the land is left fallow. Akçakent district is one of the districts where production is low, like our Boztepe district. In Akçakent district, grape production as table seed and wine grapes is in the 1st place with 67 tons, apples are in the 2nd place with 16 tons, and walnuts are in the 3rd place with 12 tons.

2.3. Status of Akpınar district fruit growing

Area and production values of fruit species produced in Akpınar district are given in Table 6.

Table 6. Production pattern of fruit species grown in Akpınar district (Anonymous, 2022b).

Species	Amount of production (tons)	Area (dec.)	Average yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	1 730	1 165	61	28 308	1 965	30 273
Apple	78	505	58	1 334	1 774	3 108
Grape	675	2 390	282	-	-	-
Pear	6	11	38	158	-	158

The total agricultural area of the district is 307 130 decares. 41 060 decares of land, which is 13.4% of the agricultural lands, is irrigated. Field crops are grown in 68.2% of the 213 809 decares of agricultural land used in the district, fruits in 1.7% and vegetables in 0.9%. 29.2% of the land is fallow. In Akpınar district, grape production has a very important place. Table grapes are grown in the district. Walnut is in the 1st place with a production of 1 165 tons, grapes is in the 2nd place with a production of 675 tons, and apples are in the 3rd place with a production of 78 tons.

2.4.The Situation of Boztepe district fruit growing

The area and production values of the fruit species produced in Boztepe district are given in Table 7.

Table 7. Production pattern of fruit species grown in Boztepe district (Anonymous, 2022b).

Species	Amount of production (tons)	Area (dec.)	Average yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	3	72	19	159	460	619
Apple	19	23	19	970	586	1 556
Grape	3	8	375	-	-	-

There are 417.460 decares of agricultural land belonging to the district. 16 850 decares of land equivalent to 4% of agricultural lands are irrigated. Field

crops are grown in 75.7% of the 401 961 decare area used, fruits in 0.1% and vegetables in 0.04%. 24.6% of agricultural land is left fallow. The district has fertile agricultural land outside the shores of Seyfe Lake. However, the irrigation possibilities of the lands are limited. The fact that the Yamula dam water will irrigate the district's agricultural lands is considered as an opportunity. It is the district where the least fruit production is made among Kırşehir districts. Apple takes the first place with 19 tons of apple production. Grapes and walnuts follow apples with 3 tons each.

2.5. The Situation of Çiçekdağı district fruit growing

The area and production values of the fruit species produced in Çiçekdağı district are given in Table 8.

Table 8. Production pattern of fruit species grown in Çiçekdağı district (Anonymous, 2022b).

Species	Amount of production (tons)	Area (dec.)	Average yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	91	840	12	7 600	14 350	21 950
Apple	369	220	90	4 100	5 250	9 350
Grape	205	1 145	179	-	-	-
Apricot	56	45	20	2 800	1 035	3 835
Almond	41	350	12	3 450	4 500	7 950
Cherry	84	35	50	1 680	235	1 915

The total agricultural area of the district is 795 080 decares, and 29 400 decares of land equivalent to 3.7% are irrigated. In 48.3% of the 677 239 decares of agricultural land used, 0.3% of field crops, 0.3 % of fruit and 0.1% of vegetables are grown and 41.3% of the land is left fallow. The most produced fruit type in Çiçekdağı district is 369 tons of apples. With a production of 205 tons, grapes are in the 2nd place and walnuts are in the 3rd place with 91 tons.

Especially the abundance of newly established grape orchards in the district draws attention.

2.6. Situation of Kaman district fruit growing

Area and production values of fruit species produced in Kaman district are given in Table 9.

Table 9. Production pattern of fruit species grown in Kaman district (Anonymous, 2022b).

Species	Amount of production (tons)	Area (dec.)	Average yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	1 781	14000	9	199 000	44 000	243 000
Apple	1 668	282	33	48 040	9 130	57 170
Grape	2 950	4 400	708	-	-	-
Apricot	111	90	27	4 970	2 080	7 050
Almond	37	320	5	7 350	7 020	14 370
Pear	545	20	60	9 080	2 650	11 730
Cherry	55	40	22	2 500	690	3 190
Plum	38	-	30	1 250	200	1 450

The total agricultural area of the district is 885 081 decares and 77 850 decares of land, which is 9.4% of the land, is irrigated. Field crops are grown in 80.7% of the used agricultural areas, fruits in 2.1%, vegetables in 0.9%, and 16.3% are left fallow. Grape is the most commonly produced fruit type in Kaman district. Table seed, table seedless and wine grapes are grown in high quantities in the district. Kaman walnut cultivation, which has an important position among walnut varieties in our country, has gained a new momentum with the establishment of new orchards. Although grape cultivation is in an important position among the cultivated species as in other districts, it is the only district where especially seedless grapes are grown. Grapes are in the 1st

place with 2 950 tons, walnuts are in the 2nd place with 1 781 tons, and apples are in the 3rd place with 1 668 tons.

2.7. The situation of fruit growing in Mucur district

Area and production values of fruit species produced in Mucur district are given in Table 10.

Table 10. Production pattern of fruit species grown in Mucur district (Anonymous, 2022b)

Species	Amount of production (tons)	Area (dec.)	Average yield per tree (kg)	Number of trees at bearing fruit (pieces)	Number of trees at non-bearing fruit (pieces)	Total number of trees (pieces)
Walnut	447	5 600	20	22 350	37 015	59 365
Apple	164	85	49	3 365	2 105	5 470
Grape	12	30	400	-	-	-
Apricot	10	35	18	600	100	700
Almond	10	28	15	670	96	766
Pear	2	-	33	60	10	70
Cherry	23	45	25	910	1 295	2 205
Plum	11	5	29	375	80	455

The agricultural lands of the district are 802 950 decares in total and 24 060 decares of land equivalent to 3% are irrigated. Of the 523 420 decares of agricultural area used, 84.8% of the field crops, 0.6% of fruit, 0.8% of vegetables are grown, 13.8% of the land is fallow. The most produced fruit type in Mucur district is walnut with 447 tons, apple is in the 2nd place with 164 tons and cherry is in the 3rd place with 23 tons. The number of newly established walnut orchards in the district has also increased significantly.

3. CONCLUSION

When the province of Kırşehir is examined in terms of its location in the world, it is located between 38°50’-39°50’ north latitudes and 33°30’-34°50’ east longitudes. Accordingly, it is seen that there is no geographical obstacle to prevent the development of fruit growing in terms of latitude and longitude for Kırşehir province. It has 7 districts: Center, Kaman, Çiçekdağı, Mucur, Boztepe, Akpınar and Akçakent. In these districts, the continental climate of

Central Anatolia prevails. Kırşehir, located in the middle of the Central Anatolian region, is among the easily accessible provinces of Turkey due to its location on the highway transportation network that goes from east to west and from south to north. Proximity plays an important role in the marketing of agricultural products. This positively affects the transit transportation of Kırşehir with many provinces. Due to the limiting effect of the continental climate seen in Kırşehir, the agricultural production value of the province is quite low and its share in the country has remained below 1%. However, no matter what the conditions are, the desired level in terms of quality and yield will be reached with a breeding using modern production techniques. Thus, more positive contributions will be provided to the people of the region and therefore to the economy of the country. Especially Kaman walnut, which is grown in the province, needs to gain the brand value it deserves. At the same time, it is necessary to determine the species and varieties suitable for the ecology of the province and to carry out adaptation studies. Thus, it is a fact that more positive contributions will be made to the people of the region and therefore to the country's economy.

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CHAPTER 12

FROM PADOVA TO YALOVA
BIOCONTROL OF THE ASIAN CHESTNUT GALL WASP IN ITALY AND TURKEY

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INTRODUCTION

The Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), is one of the four non-*Quercus* associated species of the tribe Cynipini, which induces formation of globular galls on actively growing leaves and shoots of all *Castanea* species and their hybrids (Figure 1) (Stone et al., 2002; Buffington & Morita, 2009).

This specialist chestnut herbivore, native to China, is an invasive species included in the quarantine list of the European Union (EC, 2000). After being accidentally introduced into Japan, Korea, North America, and Nepal (in the 1940s, 1950s, 1970s, and 1990s, respectively), it arrived in northwestern Italy in 2002 and then spread rapidly throughout Europe (Slovenia: 2005; France: 2005; Hungary and Switzerland: 2009; Spain, Croatia and the Netherlands: 2010; Czech Republic: 2012; Austria and Germany: 2013; Greece: 2014; Portugal: 2014; UK: 2015; Belgium: 2016) (EPPO, 2015, 2016a, 2016b; Michaelakis *et al*., 2016). It was first recorded in Turkey in the northwestern part of the country (Marmara Region-Yalova) in 2014 (Çetin *et al*., 2014).

Considering the top 10 chestnut producing countries worldwide, Turkey and Italy rank third (42,700 tone/year) and fifth (59,171 tone/year), respectively (FAOSTAT, 2012; Bento *et al*., 2016). Chestnut species are also cultivated for timber and honey production; moreover, they are important ornamental trees and essential components in food webs and evolution of forest ecosystems (Cooper & Rieske, 2010; Pezzi *et al*., 2011; Battisti *et al*., 2013). Chestnut honey is one of the most expensive types of honey in Turkey; therefore, it constitutes a significant portion of the income of many Turkish beekeepers. Chestnut honey produced in Şile area (Marmara Region), have had an international fame owing to its high chestnut pollen content (93%) (Ahmet Can, Şile Beekeepers Association, personal communication), but it is currently threatened by the high ACGW infestation levels in the region since 2016 (unpublished data).

The ACGW is commonly recognized as the most severe insect pest of chestnut species worldwide. Once arrived in a new area, it establishes successfully because it can overwinter wherever chestnut occurs (EFSA, 2010). After the establishment, populations grow and spread quickly due to females' great reproductive advantage - parthenogenesis (Nohara, 1956), and biotic and

abiotic factors that facilitates its dispersal extensively (Graziosi & Rieske, 2012; Gilioli *et al*., 2013; Guyot *et al*., 2015). Furthermore, in the invaded areas, the species experiences low levels of natural enemy pressure (Otake *et al*., 1982; Murakami *et al*., 1995; Ito & Hijii, 2000; Aebi *et al*., 2006; Quacchia *et al*., 2013; Matosevic & Melika, 2013; Panzavolta *et al*., 2013; Palmeri *et al*., 2014; Colombari & Battisti, 2016b) [see Santi and Maini (2011) for unusually high natural enemy pressure in the year following ACGW arrival].

The ACGW has a univoltine life cycle in which adults become active in early summer and oviposit into newly developed buds of chestnut trees (EPPO, 2005; Branco *et al*., 2016). Larvae hatch in 30-40 days and overwinter within the bud tissue in the first instar. In the following spring, gall tissue starts to develop around the feeding larvae to end up chambers for each larva. Larval development continues in these larval chambers through two more instars that take almost two months, a rather fast developmental pace in comparison to the first instar. Pupation occurs within the galls between late spring and early summer (EPPO, 2005; EFSA, 2010; Viggiani & Nugnes, 2010).

Its attacks strongly influence growth of the host chestnut tree, causing negative impact on fruit and wood production. ACGW galls can directly prevent the formation of flower, new shoots, and leaves, leading to a reduction in leaf area, photosynthesis, and tree biomass, which can extend into future years after the initial ACGW attack (Kato & Hijii, 1997; Battisti *et al*., 2013; Marcolin *et al*., 2017). In China, its occurrence is observed cyclically and regionally due to the regulating effects of natural enemies. Significant nut yield losses (although rising as high as 80%, usually between 15% and 30%) can be observed for two-three years, and then they are followed by only mild damage during approximately the next 10 years (Zhang, 2009; EFSA, 2010). Yield impact estimates are not available for Japan, although a threshold of 30% infestation of new shoots is indicated as the injury level (Gyoutoku & Uemura, 1985). Payne *et al*. (1983) claimed that the ACGW might have caused losses between 50% and 75% in the USA. In Italy, yield reductions of 50-75% and 20-90% were estimated for two different locations, but in the second case, losses were in combination with the nut moth, *Cydia fagiglandana* (Lepidoptera: Tortricidae) (Speranza & Paparatti, 2010; Bosio *et al*., 2013). The only quantitative data were reported in Battisti *et al*. (2013), where yield losses

in relation to gall density were calculated. During the first observations in Turkey, it was discovered that 80% of the branches on many trees had galls and the nut production almost ceased in Gacık/Yalova, the region where the pest was first recorded (İpekdal *et al*., 2014).

Following Italy, Turkey also adopted classical biological control against the ACGW and transferred the biological control agents from the University of Padova in Italy to Yalova-Turkey (İpekdal *et al.*, 2017). Currently, Italy has achieved success in ACGW management in the north, whereas Turkey still needs some more time to get the first positive results. In this review, we summarized the progression of the ACGW management programs in two countries.

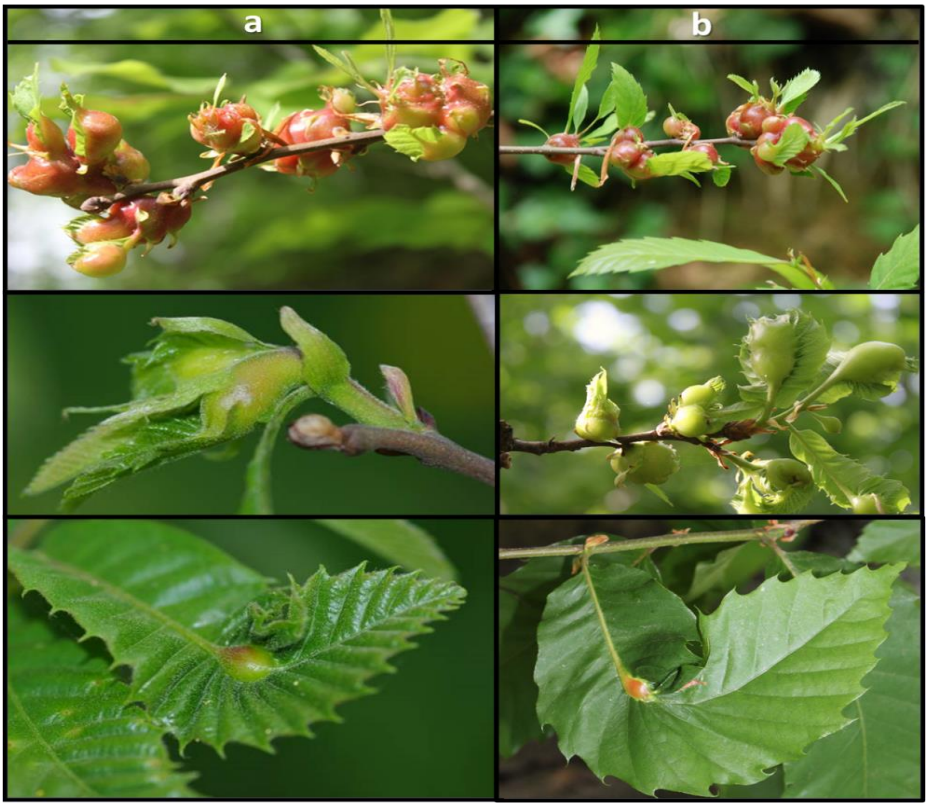

Figure 1. Different types of galls formed by *Dryocosmus kuriphilus* on chestnuts in (**a**) Veneto Region, Italy, (Photos: Fernanda Colombari) and (**b**) Yalova Province, Turkey (Photos: Kahraman İpekdal).

MANAGEMENT OF THE ACGW

Management of ACGW infestations, after several unsuccessful attempts such as insecticide applications and resistant variety trials, currently relies solely on classical biological control (Moriya *et al*., 1989). Extensive biocontrol programs have been implemented in Japan and the USA and, more recently, in Italy and Turkey through the introduction of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) (Figure 2) (Aebi *et al*., 2007; Cooper & Rieske, 2007; Quacchia *et al*., 2008; Gibbs *et al*., 2011; Colombari & Battisti, 2016b; İpekdal *et al*., 2017).

Although *T. sinensis* is one of the several parasitoids of the ACGW in its native range, studies conducted in 1970s in Japan showed that it is the most abundant one (Murakami, 1981). It has a well-synchronized phenology with the ACGW (Figure 3); it uses both visual and olfactory cues for host location that might allow it to overcome environmental complexity (Graziosi & Rieske, 2013); it has a high dispersal capacity (as a result of combination of short- and long-distance flights) (Colombari & Battisti, 2016b); and its prolonged diapause may allow it to overcome environmental changes and adverse conditions such as limited abundance and availability of the ACGW (Quacchia *et al*., 2014; Ferracini *et al*., 2015a). Altogether these traits make *T. sinensis* a successful biological control agent. Furthermore, recent studies do not report any severe effect on non-target hosts (Quacchia *et al*., 2014; Ferracini *et al*., 2015b). Consequently, a decline of ACGW populations has been reported in the years following *T. sinensis* release in Japan, the USA and Italy (Moriya *et al*., 2002; Cooper & Rieske, 2007; Ferracini *et al*., 2015b).

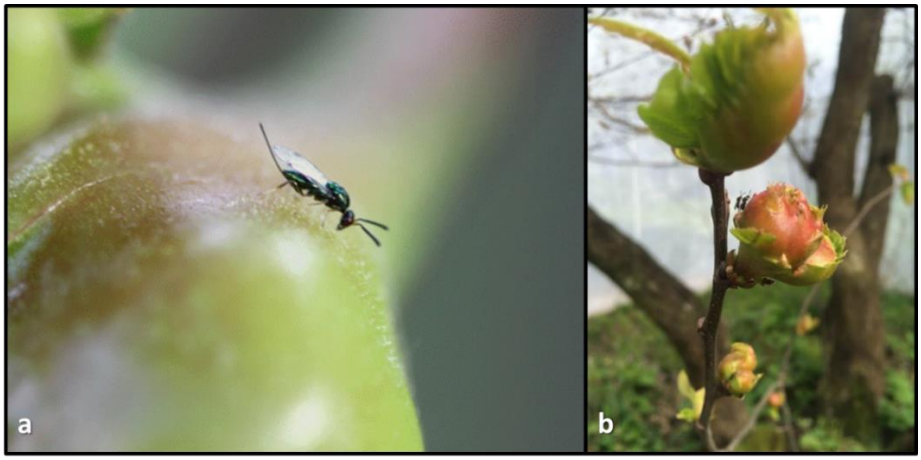

Figure 2. *Torymus sinensis*, host specific larval parasitoid of the Asian chestnut gall wasp, **(a)** adult female (Photo: Yaşar Aksu), and **(b)** adult male and female mating after being released on a chestnut gall in Turkey (Photos: Kahraman İpekdal).

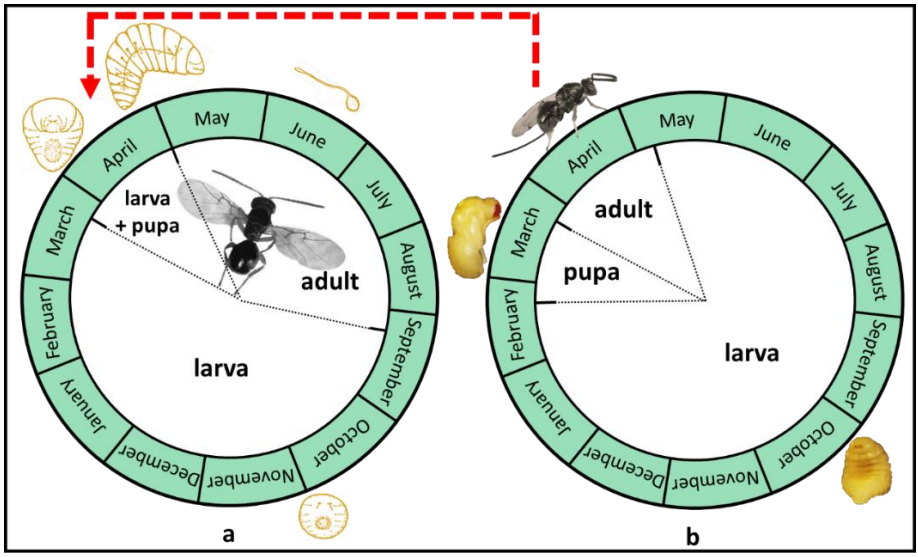

Figure 3. Life cycle of **(a)** the Asian chestnut gall wasp, *Dryocosmus kuriphilus*, and **(b)** its larval parasitoid, *Torymus sinensis*, in Turkey (adapted from İpekdal *et al.*, 2017).

MANAGEMENT PRACTICES IN ITALY

There is a long tradition of sweet chestnut cultivation in Italy because of nut and timber production. The present distribution of the species is heavily linked to past human settlements and to different management systems, according to the targeted products and services (Pezzi *et al*., 2011; Conedera *et al*., 2016). Chestnut covers an area of about 790,000 ha in the country. Despite stands for nut production represent less than 7% of the area, Italy is an important chestnut producer (Bento *et al*., 2016) and many family economies rely on chestnut production. For these reasons, the arrival of *D. kuriphilus* in 2002 in northwestern Italy put growers and managers on alert.

Immediately a classical biological control project was initiated in Piedmont Region where the pest was first recorded (Ferracini *et al*., 2015a). Positive and encouraging results gathered in the following years and the simultaneous spread of the pest throughout the country (Figure 4) led to a national biological control plan between 2010 and 2012 based on establishing regional rearing centers, such as the one in Veneto Region (Figure 5), to rear *T. sinensis* from field-collected galls (Projects LOBIOCIN and BIOINFOCAST). Regarding northeastern Italy (Veneto Region), the first release of the parasitoid (150 individuals) was made in 2010 at the site where the pest was first recorded, and other four introductions (150 individuals in each release) were made in 2011 and 2012 with parasitoids provided by the University of Turin. From 2013 to 2015, almost 500 releases were made by using the individuals produced locally by the rearing center.

In summer 2015, chamber gall occupancy was checked by dissecting a total of 1000 galls (50 galls per monitored site) and following results were found:

chambers occupied by the ACGW = 0.5%

empty chambers = 13.0%

chambers occupied by native parasitoids = 4.2%

chambers occupied by *T. sinensis* = 82.3%.

Thus, in Veneto Region, in three to five years from the first release, the biocontrol agent inflicted an average mortality up to 82%, resulting in an enhance at nut yields to about 50% of the values observed before the arrival of

the ACGW and in a decrease at pest population size under the damage threshold (Battisti *et al*., 2013).

The success of the classical biological control program in Veneto Region was achieved somewhat earlier than in other programs (Colombari & Battisti, 2016a). For example, in Japan, a decline of ACGW populations below the tolerable damage level was reported at least four years after the release of the biocontrol agent (Moriya *et al*., 2002). Ferracini *et al*. (2015a) documented a level of parasitism as high as 85.4% eight years after the releases in northwestern Italy.

Successful establishment and high reproduction occurred at all sites, independent of gall density and residence time of both the host and the biocontrol agent. The habitat structure (*i.e.*, resource distribution) likely played a fundamental role in the success of the program. Small, continuous, and clustered chestnut patches might provide considerable ACGW availability for the parasitoid during subsequent years, and abiotic factors, such as wind, facilitated the dispersal rate of the parasitoid (Colombari & Battisti, 2016b).

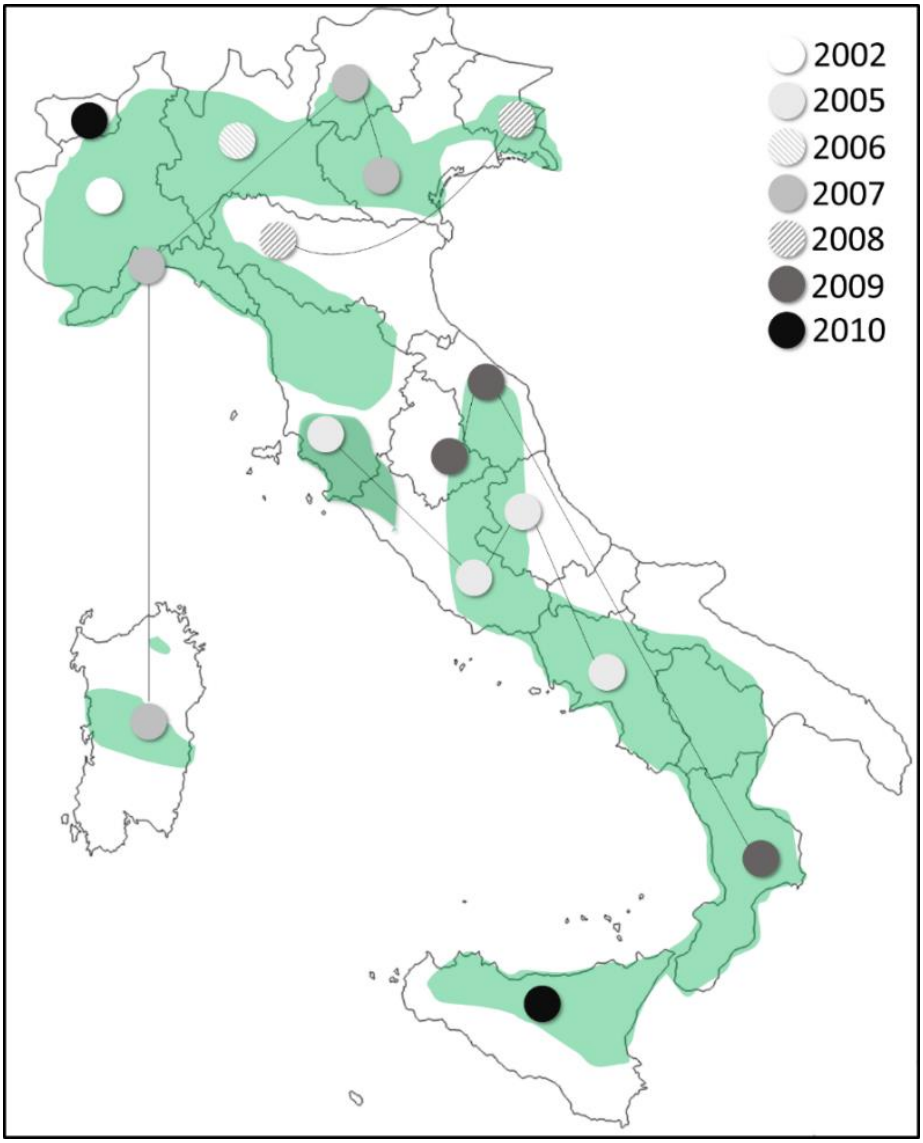

Figure 4. Distribution of the sweet chestnut, *Castanea sativa*, (green area) and range expansion by the Asian chestnut gall wasp from 2002 to 2010 in Italy.

Figure 5. *Torymus sinensis* rearing station in Veneto Region, Italy (Photos: Kahraman İpekdal).

MANAGEMENT PRACTICES IN TURKEY

Chestnut covers an area of about 262,045 ha mixed with oak, beech, and linden in Aegean (western), Marmara (north-western), and Black Sea (northern) regions of Turkey, along with 2,500 ha of private plantations especially in Aydın, Bursa, Izmir, and Manisa (OGM, 2013). In Adana, Hatay, Isparta, and Mersin less significant private gardens for nut production have also been established (Figure 6).

The chestnut growers in Yalova, where the ACGW was first recorded in Turkey, claims that the galls have been seen since 2008, however back then they had not been as dense as they were in 2014 when the pest population was high enough to be noticed by local researchers. Therefore, it could be concluded that the ACGW entered Turkey at least six (probably more) years before the first record. The growers also admitted that some of them had imported chestnut grafts from Italy in 2007 and 2008. Thus, Italy seems to be the possible origin of the ACGW in Turkey. The pest currently occurs in the entire Marmara Region several spots along the Black Sea coast, and in İzmir (Figure 6) (İpekdal *et al*., 2014; Mıcık *et al*., 2020; Mıcık and İpekdal, 2021), and it may spread

throughout the entire Black Sea and Aegean regions within a decade via active and passive dispersal along with anthropogenic transportation.

Upon the first detection of the ACGW in Turkey, *T. sinensis*-based classical biological control studies started with the knowledge and *T. sinensis* adults transported from Veneto Region through a protocol between University of Padova and the Turkish General Directorate of Forestry. A rearing and monitoring laboratory has been established in Yalova-Turkey (Figure 7). As a start, approximately 2,000 *T. sinensis* adults (emerged from ca. 7,000 galls collected from Veneto Region) were released in Gacık-Yalova in April 2015 following the gall formation in the region. In autumn of the same year ca. 15,000 galls were collected from the release site in order to monitor the establishment of the released parasitoids. However, no parasitoids emerged from the collected galls in April 2016. We concluded that the number of galls collected was not enough to give an idea about the portion of the parasitoids established after the release in 2015 (İpekdal, 2022).

A second party of ca. 8,000 dry chestnut galls were transported again from Veneto and reared in Yalova where approximately 4,200 *T. sinensis* adults emerged and were released in Yalova in April 2016 (İpekdal, 2022).

Ca. 200,000 galls were collected from the release sites in autumn 2016 – winter 2017, and kept in the rearing station in Yalova. In April 2017, approximately 550 *T. sinensis* individuals emerged from these galls which has been taken as a sign of successful establishment of the parasitoid population in the region and no further parasitoid transportation has been planned (İpekdal, 2022).

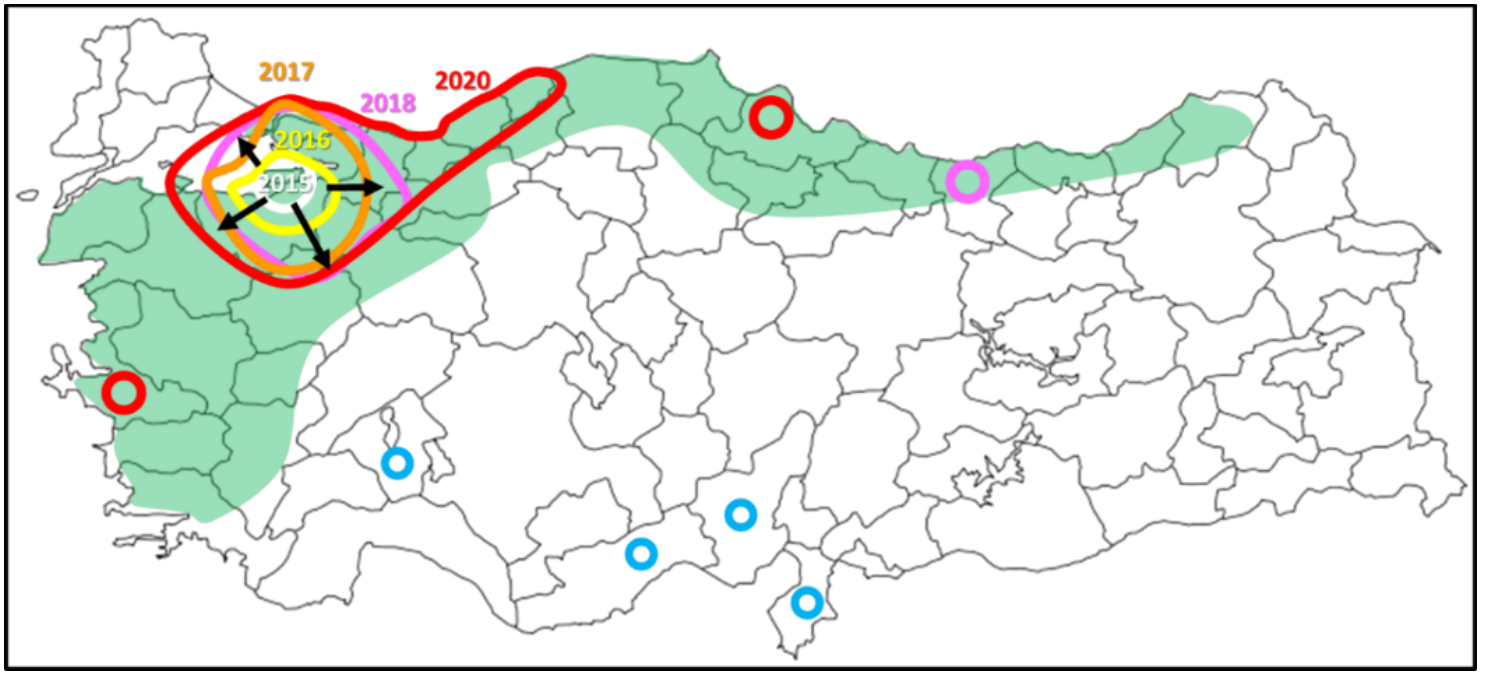

Figure 6. Distribution of the sweet chestnut, *Castanea sativa*, (green area; limited private gardens: blue circles) and range expansion by the Asian chestnut gall wasp from 2015 to 2020 in Turkey.

In autumn 2017 – winter 2018, 300,000 galls were collected from the release sites, which yielded 3,250 *T. sinensis* adults in following April. However, approximately only 100 of them could be released into the nature as they started emergence before the gall formation started in nature. This was probably due to warmer winter temperatures in 2018 than normal as reported by the Turkish Meteorological Service (TMS, 2019). Early emerging adults were released into the previously established greenhouse including chestnut seedlings infested by the ACGW. The greenhouse conditions caused galls to emerge earlier than in nature and thus provided us a backup releasing environment. Additionally, a climate chamber was established for keeping the galls in a constant temperature during the winter in order to by-pass similar temperature fluctuations in the future (İpekdal, 2022).

Figure 7. *Torymus sinensis* rearing station in Yalova, Turkey. **(a)** The outdoor booth for storing field-collected dry galls and rearing *T. sinensis* adults, **(b)** adult emergences from stored dry galls, **(c)** change chamber where dry galls are kept, **(d)** releasing mated *T. sinensis* adults in nature (Photos: Kahraman İpekdal).

In autumn 2018 – winter 2019, 344,000 galls were collected and 7,255 *T. sinensis* adults were reared (İpekdal, 2022). Approximately 1,300,000 galls were collected in autumn 2019 – winter 2020 and 1567 *T. sinensis* adults were reared in 2020 April. The reason of the drop in 2020 was probably caused by an inconvenience in the new overwintering boxes thar were tried that year since we found many dead *T. sinensis* adults in the boxes failed to collect in the collection section of the boxes. On top of it, the Covid-19 pandemic prevented us to act quickly on site. Even so, we are confident that *T. sinensis* has been established successfully in Yalova, the first release site in Turkey. A research project has recently been launched by the Turkish General Directorate of Forestry Marmara Forestry Research Institute in order to monitor the range expansion of *T. sinensis* population in Marmara Region.

FUTURE PERSPECTIVE

Introduction and establishment of an exotic insect species in a new area is one of the most intriguing topics of ecology and classical biological control, and by definition, it is not very different from biological invasions (Mack *et al.*, 2000). In this respect, biological control programs, such as those conducted against the ACGW in Italy and Turkey, harbor great potential for studying invasive populations theoretically, along with their ultimate practical aims focusing on management of these populations (Fauvergue *et al.*, 2012). Therefore, a wide range of researchers can benefit from being participated at biocontrol studies with different aspects which eventually would affect success of biocontrol efforts positively by improving the conceptual framework of the field.

Although the ACGW situation in the north of Italy seems to be under control, with production thresholds almost in line with the normal trend before the arrival of the pest, in spring 2017 some further releases were done in central-southern regions of the country (Calabria, Campania, Lazio and Sicilia Regions) where the percentage of parasitization by *T. sinensis* resulted under 50% that was recorded in 2015. Biocontrol studies continue in southern Italy and monitoring studies should continue in the north, where pest-parasitoid equilibrium has established but might not always be so due to unpredictable biotic and abiotic factors.

In Turkey, the pest is spreading rapidly in every direction and its dispersal is being monitored strictly. New forest ecosystems and plantations are being invaded on an annual basis and this creates various experimental opportunities. The importance of these opportunities is better appreciated regarding high diversity of oak galls, their parasitoids, and chestnut varieties in Turkey.

In Turkish experience, the main cause of the ACGW spread in the country to huge distances has been due more to human-mediated transportation than the pest's natural dispersal capacity. This is in concordance with Aebi *et al*. (2006) who stated that the frequent exchange of chestnut materials such as cultivars, saplings, and grafts, mainly among chestnut growers, plays an important role in spread of the pest. The fact that ACGW eggs and first instar larvae remain within the dormant buds for many months between early summer and following spring, or shortly, its asymptomatic nature, contributes greatly to human-mediated transportation of the pest (EPPO, 2005; Graziosi & Santi, 2008; Gilioli *et al*., 2013). Therefore, the importance of being in contact to local growers and beekeepers should not be ignored. We conducted a FAO-funded project aiming to raise awareness in the affected areas and to provide technical support for the ACGW management practices in Turkey in 2015 and 2016. During this project, we compiled booklets and brochures for local chestnut growers focusing on identifying the pest and actions to take for a better control. We also organized several workshops for foresters and growers. The foresters participated at the workshops still provide us regular information on the ACGW in their regions. Similar activities should be conducted more frequently along with applying stricter quarantine measures and more disincentive precautions against illegal transportation.

One of the most striking outcomes of the studies and observations on the ACGW-*T. sinensis* system so far is the difference in response of these species to the increasing temperature (Ferracini *et al.*, 2022; İpekdal, 2022). Due to this difference, climate change can cause a phenological mismatch between the host and the parasitoid, which can eventually disrupt biological control of the ACGW. Therefore, this system should be monitored carefully even after the biological control is achieved and the local populations of the ACGW seems to be kept under the economic threshold. It should be kept in mind that biological

control by using *T. sinensis* can loose its effectiveness in the near future, and ACGW populations can grow to their pre-management sizes. For this reason, research for alternative management approaches should be conducted.

Although it has been reported that the AGCW could cause tree deaths, there is not any robust evidence that confirms tree mortality (EPPO, 2005; EFSA, 2010). Nevertheless, trees can indirectly be affected by the pest as a result of being weakened by intensive attacks and they likely become highly susceptible to other factors such as drought and infection by pathogens. Recently, in different Italian chestnut orchards and stands, *Gnomoniopsis* sp. (agent of brown rot on nuts) (Diaporthales: Gnomoniaceae) have been found to be associated with the necrosis of the ACGW galls (Figure 8a), and a similar fungus infection has been observed in Turkey without any solid confirmation (Figure 8b).

Figure 8. Gall necrosis due to *Gnomoniopsis* sp. **(a)** in Italy (Photo: Giorgio Maresi), and **(b)** in Turkey (Photo: Kahraman İpekdal).

A recurrence of damage due to *Cryphonectria parasitica* (Murr.) (Diaporthales: Cryphonectriaceae), responsible for the chestnut blight, has also been observed on highly ACGW-infested trees in Italy (Magro *et al*., 2010; Turchetti *et al*., 2012; Maresi *et al*., 2013). The chestnut blight has been known to occur in Turkey since 1967 (Akdoğan & Erkam, 1968) and promising results have been obtained from biological control practices recently (Çeliker & Onoğur, 2011; Çeliker *et al*., 2017). Although there is not any observation on the recurrence of chestnut blight damage in the current range of the ACGW in Turkey, yet it and other possible threats that can be caused by pathogenic fungi should also be monitored.

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CHAPTER 13

PROBIOTICS: HEALTH BENEFITS

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INTRODUCTION

The expression of probiotic is acquired from a Greek word meaning “for life” and is used to describe microorganisms that, when consumed in sufficient amounts, benefit the health of the host. (Roberfroid, 2000; FAO/WHO, 2001; Senok et al., 2005; Soccol et al., 2010; Chapman et al., 2011; Hill et al., 2014; Pandey et al., 2015; Sanders et al., 2018; Plaza-Diaz et al., 2019). The detection of the existence of probiotic microorganisms is thought to be based on the Nobel Prize-winning Russian scientist Elie Metchnikoff in the early 1900s, who stated that Bulgarian villagers consume fermented milk products as the secret of their long and healthy life (Metchnikoff, 1908; Gupta and Garg, 2009) The definition of “probiotic” was first used by Lilly and Stilwell in 1965. (Lilly and Stillwell, 1965). Then, it was defined by Fuller as “non-pathogenic microorganisms that, when consumed, exert a positive effect on the health of the host” (Pandey et al., 2015). Probiotics are explained by the Food and Agriculture Organization of the United Nations (FAO) and the World Health Organization (WHO) as "live microorganisms that confer health benefits when administered in adequate amounts to the host" (FAO/WHO, 2001; Hill et al., 2014; Pandey et al., 2015; Staniszewski and Kordowska-Wiater, 2021). This definition is an endorsement by FAO/WHO, organized by ISAPP (International Scientific Association of Probiotics and Prebiotics) and convened to discuss the modern significance of the definition of probiotic expression, and published in 2001 by the consensus panel (FAO/WHO, 2001; Sanders et al., 2018). Probiotics are micro-organisms that can grow at 37°C, are not affected by the digestive system conditions, and can progress to the intestine, and have positive effects on the health of the host by regulating the microbiota (Fijan, 2014; Staniszewski and Kordowska-Wiater, 2021). Specific probiotic strains of *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Escherichia*, *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* bacterial genera are given as examples of probiotic microorganisms. Also, *Saccharomyces* is the only yeast genus proven to be probiotic (Gibson and Roberfroid, 1995; Jin et al., 2000; Alvarez-Olmos and Oberhelman, 2001; Czerucka et al., 2007; Gupta and Garg, 2009; Fijan, 2014). The 2 most common types of probiotic bacteria used in foods are *Bifidobacterium* and *Lactobacillus*. Strains of these microorganisms are resistant to acidic conditions of the stomach, pancreatic enzymes and bile salts. It also has the ability to bind to the mucosa and colonize the

gastrointestinal tract (Martins et al., 2014). Although *Enterococcus* and *Escherichia* are defined as probiotics, there are still concerns about the safety of these bacteria (Donohue and Salminen, 1996; Rolfe, 2000; Eaton and Gasson, 2001; FAO/WHO, 2001; Holzapfel et al., 2001; Ishibashi and Yamazaki, 2001; Senok et al., 2005).

1. THE HISTORY OF PROBIOTICS

The origin of dairy products (kefir, kumiss, leben etc.) dates back to ancient times (Howells, 1992). These products, which are still consumed today, were used medically before the existence of probiotic bacteria was discovered (Shortt, 1999). Elie Metchnikoff, a Nobel Prize-winning scientist in Medicine at the Pasteur Institute, reported in 1908 that the secret to a healthy and long life is consuming yogurt (FAO/WHO, 2001; Soccol et al., 2010). Metchnikoff stated that microorganisms, *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, which constituent the natural microflora of yogurt, suppress pathogenic microorganisms in the intestine; He attributed the longevity of Bulgarian villagers to their consumption of yogurt (Metchnikoff, 1908). In the same years, Henry Tissier, a French pediatrician, discovered bifidobacteria in breastfed infants, suggesting that administering bifidobacteria to patients with diarrhea helps restore healthy intestinal flora (FAO/WHO, 2001; Soccol et al., 2010). Metchnikoff and Tissier gave the first scientific advice on the use of probiotics for bacteria (FAO/WHO, 2001). In the early 1930s, in Japan, Shiro studied bacteria that can survive in the gut, and the product containing *L. acidophilus* Shirota became the foundation of the Yakult Honsha Company (Soccol et al., 2010). Fuller redefined the term of probiotic in 1989 to draw attention to the microbial nature of probiotics and defined it as "live microbial food supplements that beneficially affect the host's intestinal balance by improving it". In 1992, Havenaar and Huis in't Veld made a similar explanation for probiotics. In the last 20 years, studies in the field of probiotics have progressed considerably and significant progress has been made in the selection, characterization, consumption of specific cultures (FAO/WHO, 2001). The guide stating the qualifications required for a product to be called a probiotic was published by FAO/WHO in 2002 (FAO/WHO, 2001).

2. PROBIOTIC BACTERIA

Probiotic bacteria consist of strains of *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, *Streptococcus* bacteria, also known as lactic acid bacteria (lab), and non-lab bacteria and yeasts (Vinderola et al., 2000; O'Sullivan, 2001; Soccol et al., 2010; Al-Nabulsi et al., 2014). The commonly used species among these bacteria are strains of *Bifidobacterium*, *Lactobacillus* and *Enterococcus* genera (Holzapfel et al., 1998; Shah, 2007; Rodgers, 2008; Al-Nabulsi et al., 2014; Martins et al., 2014). Strains belonging to the *Bifidobacterium* and *Lactobacillus* genera are frequently isolated from the gastrointestinal tract of healthy people. For this reason, strains belonging to the *Bifidobacterium* and *Lactobacillus* genera are generally used worldwide (Shah, 2007; Saulnier et al., 2009; Champagne et al., 2011; Martins et al., 2014).

Microorganisms considered as probiotics are shown in Table 1 (Goldin, 1998; Klaenhammer and Kullen, 1999; Holzapfel et al., 2001; Krishnakumar and Gordon, 2001; Chow, 2002; Playne et al., 2003; Shah, 2004; Shah, 2007; Soccol et al., 2010; Patel and Denning, 2013; Martins et al., 2014; Dixit et al., 2016; Kerry et al., 2018; Zheng et al., 2020).

Table 1. Microorganism considered as probiotics.

Lactobacillus species	***Bifidobacterium* species**	Other lactic acid bacteria	**Nonlactic acid bacteria**
Lacticaseibacillus casei subsp. *Immunitas* (formerly named *Lactobacillus casei*)	*B. adolescentis*	*Enterococcus faecalis*	*Akkermansia muciniphila*
Lacticaseibacillus paracasei (formerly named *Lactobacillus paracasei*)	*B. animalis*	*Enterococcus faecium*	*Bacillus cereus* subsp. *toyoi*
Lacticaseibacillus rhamnosus (formerly named *Lactobacillus rhamnosus*)	*B. bifidum*	*Lactococcus lactis*	*Bacillus lactis*
Lacticaseibacillus casei subsp. *Shirota* (formerly named *Lactobacillus casei*)	*B. breve*	*Leuconstoc mesenteroides*	*Bacillus laterosporus*
L. acidophilus	*B. catenulatum*	*Pediococcus acidilactici*	*Bacillus subtilis*
L. amylovorus	*B. essencis*	*Pediococcus pentosaceus*	*Bacteroides uniformis*
L. crispatus	*B. infantis*	*Sporolactobacillus inulinus*	Escherichia coli strain nissle

L. delbrueckii subsp. *bulgaricus*	*B. lactis*	*Streptococcus salivarius*	Peptostreptococcus productus
L. delbrueckii subsp. *lactis*	*B. laterosporus*	*Streptococcus sanguis*	*Propionibacterium freudenreichii*
L. gallinarum	*B. longum*	*Streptococcus thermophilus*	*Propionibacterium jensenii*
L. gasseri			*Saccharomyces boulardii*
L. helveticus			*Saccharomyces cerevisiae*
L. johnsonii			
L. plantarum			
L. reuteri			
Limosilactobacillus fermentum (formerly named *Lactobacillus fermentum*)			
Ligilactobacillus salivarius (formerly named *Lactobacillus salivarius*)			

The strains most commonly included in probiotic food products are *Lacticaseibacillus casei*, *Lacticaseibacillus rhamnosus* (formerly named *Lactobacillus rhamnosus*), *Lacticaseibacillus casei*, *Lactobacillus* (*L.*) *acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, *L. plantarum*, *L. reuteri*, *Bifidobacterium adolescentis*, *B. bifidum*, *B. breve*, *B. infantis*, *B. longum*, *Enterococcus faecium*, *Streptococcus thermophilus* (Sanders, 2003; Cencic and Chingwaru, 2010; Soccol et al., 2010; Awaisheh, 2012; Martins et al., 2014).

Probiotic bacteria strains to be added to food must have some characteristics. In order to show an effective probiotic feature, the strains must be of human origin. In addition, the strains should be able to survive despite gastric acidity and bile salts, colonize in the gastrointestinal tract and adhere to the intestinal mucosa, exhibit antioxidative properties, and withstand processing and storage conditions (Conway et al., 1987; Havenaar et al., 1992; Dunne et al. 2001; O'Sullivan, 2001; Soccol et al., 2010; Al-Nabulsi et al., 2014). Even if they belong to the same bacterial species, differences in probiotic properties can be observed between strains. This causes the immunogenic effects of the strains, in other words, the effects on human health to be different (Martins et al., 2014).

3. NOVEL SPECIES

Bifidobacteria, lactobacilli and other lactic acid-producing bacteria are probiotics that are isolated from fermented milk products and the fecal microbiome. Depending on the expansion of the human microbiome and developments in genome sequencing methods, the detection of new potential probiotic microorganisms may occur (O'Toole et al., 2017; Veiga et al., 2020). Interest in the probiotic potential of *Akkermansia muciniphila*, *Bacteroides* spp., *Eubacterium* spp., *Faecalibacterium prausnitzii*, *Roseburia intestinalis* isolated from the human intestine is increasing day by day (Brodmann et al., 2017; O'Toole et al., 2017). These bacteria can perform physiological functions that are not always directly delivered by bifidobacteria or lactobacilli, such as the production of butyrate, propionate, and other bioactives (Blaak et al., 2020). Among the species with the potential to have probiotic properties, *A. muciniphila* is one of the most promising candidates. In the study carried out with the isolation of *A. muciniphila* in 2004, results were found that this bacterium is safe for use in humans and improves various metabolic parameters (Derrien et al., 2004; Depommier et al., 2019). Studies on the safety of new strains and strains thought to be probiotics for the development of new probiotic products are increasing day by day (Brodmann et al., 2017; O'Toole et al., 2017).

4. HEALTH BENEFITS OF PROBIOTICS

Digestible live microorganisms, known as "probiotics", have a very important place in nutraceutical science because they have various positive effects on the health of people who consume them (Patel and Goyal, 2013; Tegegne and Kebede, 2022). Probiotics are microorganisms that regulate intestinal health and balance, prevent diarrhea, improve cardiovascular health, regulate the immune system, can be consumed for individuals with lactose intolerance and food allergy, and have anticarcinogenic effects (Yadav et al., 2022).

The human gut microflora is the habitat for many microorganisms with beneficial, harmful and neutral effects. The ratio of beneficial microorganisms to harmful ones in the intestine has an impact on human health. Studies show that intestinal health and related human health can be improved by maintaining the balance in the intestinal microflora (Reid, 2008; Al-Nabulsi et al., 2014).

Ulcerative colitis is a common gastrointestinal disease. Studies show that probiotics improve intestinal mucosal barrier function and immune system function, prevent the growth of harmful bacteria in the intestine, and have antibacterial and anti-inflammatory effects. The use of probiotics is increasing day by day, especially in the treatment of colitis (Verna and Lucak, 2010; Shen et al., 2018; Damian et al., 2022).

Probiotics are generally regarded as functional foods. They involve strains of lactobacilli that provide health benefits. Probiotics are anti-pathogens and have therefore been recommended for the treatment of diarrhea (Goldin, 1998; Hempel et al., 2012; Al-Nabulsi et al., 2014; Damian et al., 2022).

It has been reported in studies that negative changes in the intestinal microflora cause an increase in food allergy. The interaction between the microbiota and the host is effective in the regulation of the immune system. The development of the immune system and gut microbiota takes place early in life. Exposure to maternal microorganisms through vaginal delivery is important in maintaining a strong immune system. Additionally, the intestinal flora of the baby may change due to antibiotic consumption during breastfeeding and the risk of allergic disease may increase significantly (Jakobsson et al., 2014; Damian et al., 2022). It has been determined that individuals with food allergy consume functional foods supplemented with probiotics and decrease in allergy levels, and probiotics have protective and therapeutic effects against food allergies (Goldin, 1998; Purchiaroni et al., 2013; Kerry et al., 2018; Rothenberg et al., 2019; Shu et al., 2019; Damian et al., 2022).

Some gastrointestinal tract infections caused by bacteria are common. Sometimes complications are seen due to antibiotic resistance during the treatment process. This is seen in multi-drug resistant bacteria such as *Helicobacter pylori*, which is found in diseases such as ulcers and stomach cancer (Lesbros et al., 2007; Damian et al., 2022). It has been proven by studies that consumption of foods with different functional properties, such as fruit juices fortified with probiotics and antibiotics, is beneficial in the treatment of infection (Goderska et al., 2018; Damian et al., 2022).

High serum cholesterol level is associated with coronary heart disease. In many animal studies, there is evidence that probiotics have a hypocholesterolemic effect (Xiao et al., 2003; Awaisheh et al., 2013; Al-Nabulsi et al., 2014).

More than half of the world's population has difficulty digesting lactose due to the low level of lactase enzyme in their bodies. This causes symptoms such as cramping, bloating, and diarrhea. Probiotics are bacteria and yeasts that play an active role in the gastrointestinal microbiota. Most of these microorganisms are lactic acid bacteria and have the enzyme lactase, which fragmentation the lactose present in foods. For this reason, the enzyme obtained from lactic acid bacteria helps to cure lactose intolerance (Goldin, 1998; Kechagia et al., 2013; Purchiaroni et al., 2013; Al-Nabulsi et al., 2014; Oak and Jha, 2018; Leis et al., 2020; Damian et al., 2022).

Cancer is a deadly disease that occurs due to the uncontrolled proliferation of abnormal cells. Although some treatments are applied to cancer patients, there is no definitive treatment yet. The metabolically active or inactive form of probiotics and their metabolites (ferrichrome, nucleic acid, polysaccharides, protein inhibitory compounds and SCFAs) are a natural and effective treatment method that prevents the propagation of cancer cells (Goldin, 1998; Yu and Li, 2016; Kerry et al., 2018; Legesse Bedada et al., 2020; Damian et al., 2022).

There are different mechanisms in the anti-carcinogenic activities of probiotic microorganisms. These can be listed as reducing tumor cell proliferation, inhibiting the production and activity of carcinogenic enzymes that convert procarcinogens produced by saprophytic microorganisms into carcinogens, neutralizing mutagenic substances, increasing the immune response against tumor tissue and binding toxins (Rafter, 2003; Commane et al., 2005; Roy et al., 2009; Al-Nabulsi et al., 2014).

The relationship between the composition of the gut microbiota and metabolic diseases such as obesity and diabetes has been suggested by Larsen et al.. As a result, increasing the beneficial microbiota with the use of probiotics is predicted to play an important role in neutralizing the disorder (Larsen et al., 2010).

The coronavirus disease 2019 (COVID-19) spread very rapidly at the beginning of 2020, affecting everyone around the world. Bottari et al. (2021) stated in their study that the possible benefits of probiotic application on COVID-19 infection may be related to the effect of innate and acquired immunity. Nguyen et al. (2022) stated in their study that the metabolites of *Lactobacillus plantarum*, a probiotic species, can have an antiviral effect

against COVID-19.

Most probiotic microorganisms are safe, however, caution should be exercised when administering to seriously ill or immunocompromised patients (Horwitch et al., 1995; Syndman, 2008; Islam, 2016). The most common repercussion of probiotics are gas, nausea, constipation, rash, hiccups, and infection (Islam, 2016).

5. PROBIOTICS IN FOOD SECTOR

Today, the variety of food products containing probiotic strains is quite large and increasing day by day depending on consumer demands. The development of functional foods is ensured with probiotic bacteria added to the formulation during the production of food products. The main probiotic products available in the market are dairy products such as kefir, cheese (tulum cheeses), ice cream, buttermilk, milk powder and yogurt. In addition, there are plant-based (such as kimchii, a traditional Korean dish), fruit-based, grain-based (products made with sourdough) and meat-based functional products with probiotic properties (Stanton et al., 2001; Sangwan et al., 2011; Kechagia et al., 2013; Park et al., 2014; Min et al., 2019; Nagaoka, 2019; Özkan et al., 2021; Damian et al., 2022). During the production of functional foods produced by adding probiotic strains, attention should be paid to issues such as compatibility of the product with the microorganism, the survival of the microorganism in the processing, packaging and storage processes of the food, as well as food safety. For example, the pH of the product has a great importance on the growth of the added probiotic strain (Gardiner et al., 1998; Kechagia et al., 2013).

Technological innovations used in the food industry (e.g. microencapsulation) provide new options for consumer demand as well as overcoming the stability and growth problem of probiotic microorganisms (Kechagia et al., 2013).

6. CONCLUSION

Although the term probiotic was first revealed in the early 1900s, scientific research on its relationship with health still continues. Probiotics show an antagonistic effect against certain microorganisms and improve the immunity of the host. Studies show that probiotics can be used in the treatment

of some health problems (such as prevention and treatment of gastrointestinal disorders, prevention and treatment of diabetes, prevention and treatment of allergies, regulation of the immune system, reduction of depression and cancer effects). Although the probiotic products available today are mostly in food and food supplement formats, it is anticipated that they can be produced as drugs for the disease in the near future. Studies on the detection of new probiotic strains, the evaluation of their usability in clinical applications, the identification and use of species that have positive effects on human health are thought to be the focus of the near future.

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CHAPTER 14

MAGNETIC NANOPARTICLES AND GREEN CHEMISTRY APPROACH

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1. MAGNETIC NANOPARTICLES

The rapid progress of nanotechnology has resulted in the emergence and development of many new application areas. Nanoparticles have an essential role in the emergence of these applications. Nanoparticles (NPs) are materials with characteristics with an average size between 1 and 100 nm. Insomuch that nanometer-sized ultra-small particles have different and richer physicochemical properties than macro-sized particles. These enriched properties have led to the advancement of materials science and the use of these particles in applications for the benefit of society (Ali et al., 2021).

Magnetic nanoparticles have an important place in these applications due to their unique magnetic properties.

Interdisciplinary studies on using and preparing magnetic nanoparticles have increased in recent decades. Different kinds of magnetic nanoparticles can be prepared, such as single metal nanoparticles (Fe, Co, Ni), metal alloys (CoPt, FePt), ferrites (MFe_2O_4, M = metal,) and iron oxides (Fe_3O_4, γ-Fe_2O_3). These magnetic particles can have different shapes, sizes, and crystallinity by optimizing using various methods. This diversity changes and determines their toxic properties, magnetism, physicochemical properties, magnetism, and surface properties. Thanks to these possibilities, the desired magnetic nanoparticles are prepared following the application purpose (Ansari et al., 2022).

1.1 Compsitions of Magnetic Nanoparticles

1.1.1 Single-type metal MNPs

Such nanoparticles (Fe, Ni, Co MNPs) are important, especially for electronic devices, catalysts, and sensor applications, thanks to their high magnetic properties and purity. However, producing such particles is quite laborious and costly; also, these particles are not stable in an air medium for a long time. What needs to be done is to obtain pure metallic MNPs of desired shape and size by providing an oxygen-free environment (Nguyen et al., 2021)

1.1.2 Iron Oxides and Ferrites

It is the most widely used and synthesized magnetic nanoparticle type. This is because they are more stable, less toxic, and more accessible to produce

than pure metallics(Ali et al., 2021). Iron oxide nanoparticles are generally preferred in studies because they can be synthesized superparamagnetically by simple and inexpensive methods. The different types of iron oxide with different properties (size and shape) are; Fe_3O_4 (magnetite), α-Fe_2O_3 (hematite), γ-Fe_2O_3 (maghemite,), β-Fe_2O_3 and ε-Fe_2O_3) etc. When synthesized in small sizes (less than 15 nm), magnetite exhibits both superparamagnetic and ferrimagnetic properties, while the others have weak ferrimagnetic, ferromagnetic, and antiferromagnetic properties. Therefore, magnetite nanoparticles are preferred(Wu et al., 2008).

Ferrite nanoparticles are obtained when different metals are embedded in iron oxide nanoparticles while being synthesized. The most common ones are cobalt ferrite, manganese ferrite, and nickel ferrite. Both types can be synthesized and optimized using simple methods. If it is necessary to choose between iron oxide and ferrite particles, the needs of the application should be considered. For example, iron oxide nanoparticles have lower coercivity (Hc) than cobalt ferrite nanoparticles, making them undesirable candidates for magnetic hyperthermia applications (Dutz et al., 2020).

1.2 Preparation methods

Different disciplines (physics, chemistry, biology, and materials) have developed methods to produce magnetic nanoparticles. Although it is possible to find hundreds of improved methods in the literature, a detailed examination is required to obtain the particle with the desired properties. Because they do not all involve uniform nanoparticle synthesis, they have been specified to obtain particles with different sizes, compositions, surface properties, and crystal structures. Shukla et al., in their study, divided the magnetic nanoparticle synthesis methods into three classes, as shown in Figure 1. (Shukla et al., 2021).

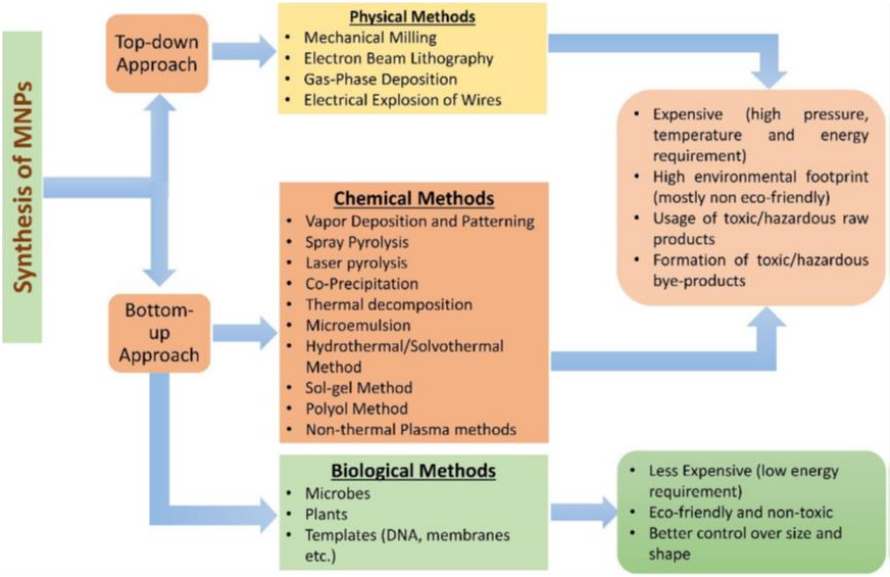

Figure 1 : Classification of synthesis methods for MNPs and their key features(Shukla et al., 2021)

In the top-down approach, nanoparticles are obtained by breaking up bulk micro-sized materials into small pieces (nano size). In contrast, in the bottom-up approach, the formation of nano-sized particles by combining atoms and molecules is defined. While the top-down approach is generally used in physical methods in magnetic nanoparticles, the bottom-up approach is used in chemical and biological processes (Abid et al., 2022).

1.2.1 Physical methods

Although the mechanical grinding method is very cheap and practical, the coercivity and magnetic saturation of the MNPs obtained are low. Likewise, the electron beam lithography method, in which iron oxide nanoparticles are obtained by splitting the iron into pieces using an electron beam, is low-cost and effective. In the vapor deposition and patterning method, the material of nanoparticles is formed by filling the voids on the films developed by vapor deposition techniques such as electrodeposition and laser ablation. In general, physical methods, although effective and easy to optimize, require certain specific equipment and materials(Shukla et al., 2021) .

1.2.2 Chemical methods

Synthesis of magnetic nanoparticles using chemical methods is preferred over other methods. The reason for this is that optimization studies are carried out with simple experimental methods, and geometric properties such as size and shape can be adjusted(Tartaj et al., 2003) One of the advantages of chemical synthesis is that the surface can be modified optionally and purposefully, providing hydrophilic and hydrophobic particles. In addition, materials and devices used in chemical methods are more accessible and less costly (Sumaira Nosheen et al., 2021a).The most commonly used methods are co-precipitation, thermal decomposition, polyol, and hydrothermal processes. Brief comparisons of the methods are given in the table shown in Figure 2. (Lu et al., 2007). In addition, the details of some methods will be briefly summarized below.

Synthetic method	Synthesis	Reaction temp. [°C]	Reaction period	Solvent	Surface-capping agents	Size distri-bution	Shape control	Yield
co-precipitation	very simple, ambient conditions	20–90	minutes	water	needed, added during or after reaction	relatively narrow	not good	high/ scalable
thermal decom-position	complicated, inert atmosphere	100–320	hours–days	organic compound	needed, added during reaction	very narrow	very good	high/ scalable
microemulsion	complicated, ambient conditions	20–50	hours	organic compound	needed, added during reaction	relatively narrow	good	low
hydrothermal synthesis	simple, high pressure	220	hours ca. days	water-etha-nol	needed, added during reaction	very narrow	very good	medium

Figure 2: Table of comparison synthesis methods(Lu et al., 2007)

The co-precipitation method is quite simple, it is based on the controlled oxidation of water-soluble Fe^{+3} and metal salts (such as Co^{+2}, Ni^{+2}, Mn^{+2} and Fe^{+2}) in an essential and inert environment, and magnetite, meghemite, ferrite nanoparticles are obtained. Although this method can be applied quite easily, optimization studies are limited. Only in the spherical 10-50 nm range hydrophilic particles with heterogeneous size range are produced. The fact that it does not contain any surfactant reduces the stability of the particles and they agglomerate.(Sumaira Nosheen et al., 2021b), (Xu et al., 2014).

Thermal decomposition method is one of the most used methods among chemical methods. It withstands the degradation of iron acetylacetonate salts in a high boiling solvent in the presence of surfactant at high temperature(Glasgow et al., 2016). This method uses long-chain surfactants such as oleic acid, oleylamine, lauric acid, and solvents such as benzylether,

octadecene and dioctyl ether (with a boiling point close to 300 degrees) are used. Optimization studies are carried out in an oxygen-free environment, in a closed, isolated system, by making a step-by-step temperature change or by varying the precursor: surfactant ratio. As a result of these optimization studies, monodisperse, superparamagnetic, and highly stable magnetic nanoparticles having various shapes and sizes are obtained. One of the most important disadvantages of this method is that the particles obtained are hydrophobic, so it is especially suitable for biomedical applications. However, this difficulty has been overcome by hydrophilic surface coating (such as silica and polymer) studies (Batlle et al., 2022), (Shukla et al., 2021), (Lu et al., 2007), (Tartaj et al., 2003).

In the hydrothermal or solvothermal method, metal salts are obtained by reacting with certain surfactants in suitable solvents at high temperatures (130ºC to 250ºC). Unlike the other two methods, high pressure (0.3 – 4 mega Pascal (MPa)) is used in this method. A Teflon-coated stainless steel container pressurized in the autoclave is required as equipment. Both hydrophilic and hydrophobic stable magnetic nanoparticles with narrow size range, monodisperse, desired shape, and size can be synthesized (Sumaira Nosheen et al., 2021a), (Lu et al., 2007).

In the polyol method, magnetic nanoparticles are formed by the reduction of metal salts in polyols such as ethylene glycol at high temperatures at a specific time interval. It is a straightforward, optimized, and effective method, the particles obtained have hydrophilic properties. During the reduction, the diol intermediate of metals is obtained, and then metal nanoparticles are obtained from this intermediate. Since polyols are used as solvents instead of water, problems such as hydrolysis are not experienced. Particles are highly resistant to oxidation as they have hydroxyl groups on their surface (Sumaira Nosheen et al., 2021a), (Shukla et al., 2021), (Tartaj et al., 2003) .

1.2.3 Biological Methods

Magnetic nanoparticles can also be synthesized using biological agents which cover fungi, bacteria, viruses, plants, etc. While biological methods are still developing, this development has accelerated with the popularization of the green chemistry approach. Because without using organic solvents and toxic chemicals, it is aimed to prepare magnetic nanoparticles that use biocompatible

wastes in the natural environment or whose production does not cause toxic waste. The mechanism of some of the methods using enzymes and bacteria is still unclear and needs to be investigated(Shukla et al., 2021)., (Ali et al., 2021)

Sustainability and green chemistry principles need to be perceived in order to understand the importance and details of biological synthesis methods. Environmentally friendly magnetic nanoparticles prepared with studies in this field will be used for the benefit of humanity as in the previous classical methods.

2. Green Chemistry And Sustainibility

Green chemistry is defined as eliminating the use of hazardous substances in chemical processes applied in material production and preventing the formation of toxic waste at the end of the process. Green chemistry does not only cover harmful substances but also aims to minimize energy and water-solvent consumption in chemical processes (Beach et al., 2009).

There is a goal-effect link between green chemistry and sustainability. Sustainability can be defined as ensuring the continuity of the existing without deterioration and decrease. Humanity must continue producing life without harming the air, water, vegetation, climate, and vitality for a sustainable world. With the increasing industrialization and population in the last century, climate change, the need for food and energy, the depletion of resources, and environmental pollution, the chemistry industry, which is at the center of production, to turn into green chemistry with the understanding of sustainability (Anastas & Lankey, 2002).

2.1 History of Green Chemistry

In 1991, the term green chemistry was first defined by Poul .T Anastas in a program organized by the US Environmental Protection Agency (EPA) to implement sustainability in the chemical and chemical industry. In order to raise social awareness, competitions in the field of greenery were organized by the US government in 1995 and 1996, working groups were formed, and books and magazines were published . In Europe, 60% of the public has a negative view of the chemical industry, which was explained in a study conducted by the European Chemical Industry Council (CEFIC) in 1994 (Clark, 1999). To reduce the negative prejudices of the public against the developing chemical

industry and more importantly for a sustainable environment, scientists have started to develop green chemistry methods for use in the industry. In 1998, Anastas and Warner published the twelve principles of green chemistry(Anastas PT, 1998). These principles describe the requirements for the design of chemical processes in accordance with the green chemistry approach.

2.2 12 Principles of Green Chemistry

The principles to be considered while designing green chemistry processes are given below(Gür & Karagölge, 2016).

1.Prevention: Waste generation should be reduced to a minimum.
2. Atom economy: Processes should be designed in such a way that all materials used are converted into products.
3. Less dangerous chemical syntheses: The designed processes should cause less toxicity to living things.
4. Designing of safer chemicals: Chemical products should be less harmful
5. Safer solvents and auxiliaries: Solvents and auxiliaries should be used as little as possible.
6. Increase energy efficiency: Energy should be used efficiently in processes, such as room temperature and pressure
7. Renewable raw materials: Economically and environmentally renewable raw materials are used in the processes.
8. Reducing chemical derivatives: Unnecessary derivatizations, such as the use of blocking groups, should not be used in processes.
9. Use of Catalyst: The use of catalyst in the processes should be increased.
10. Degradable products should be produced: After the materials are used, they should be destroyed by decomposing in a way that does not harm the environment.
11. Use of real-time analysis: Real-time analytical methods should be developed to prevent contamination that may occur during the analysis.
12. Reducing the risk of an accident: The chemicals used in the designed chemical processes must be safe to prevent accidents such as

explosion, fire, and release.

In summary, these twelve principles describe the need to design a safer environment for chemical processes, both personally and environmentally. It is aimed that there will be no adverse effects on the world and living life before and after production.

2.3 Magnetic Nanoparticles and Green Chemistry

Using the green chemistry approach, magnetic nanoparticles can be synthesized by developing methods where waste is consumed, reduced toxic effects, and minimized solvent usage. Biological methods among the synthesis methods of magnetic nanoparticles are suitable for this approach. According to the green chemistry approach, nanoparticle synthesis should be designed in the aqueous medium without using highly reactive intruders such as $NaBH_4$ and the stabilizer, which have toxic effects on the environment(Rico Martínez, 2019) .

Green chemistry methods are more compatible with sustainability principles than the chemical and physical methods of magnetic nanoparticle preparation. Chemical methods use toxic substances such as sodium borohydrate, sodium dedosyl sulfate, hydrazine, which are harmful to the environment and human health, and their wastes are formed. In physical methods, mechanical grinding systems are used according to the top-down approach, which causes high energy consumption. In the Green synthesis approach (biological methods), magnetic nanoparticles are synthesized using natural origin substances (such as vitamins and sugar), biocompatible degradable polymers (proteins, etc.), plants and microorganisms as reducing and stabilizing agents. In addition, while synthesizing nanoparticles, minimum energy consumption is ensured and no toxic waste results.(Priya et al., 2021) In particular, the synthesis of magnet nanoparticles based on iron and iron oxide 1.) plant extracts and 2.) microorganisms are used.

2.3.1 Plant Extract Based Green Synthesis

With science's development, plants' components began to be recognized. Molecules having antioxidant properties such as terpenoids, phenols, saponins, amino acids, flavonoids, proteins, and polyphenols in the content of plants serve

as a capping and reducing agent in the formation of magnetic nanoparticles(Fahmy et al., 2018) Since the plants' flowers, leaves, seeds, shells, roots, etc. are rich in these antioxidant molecules, the whole plant can be used during the procedure (Priya et al., 2021).Shape and size optimizations of magnetic nanoparticles can be made by changing the plant type and extract concentration (Fahmy et al., 2018).

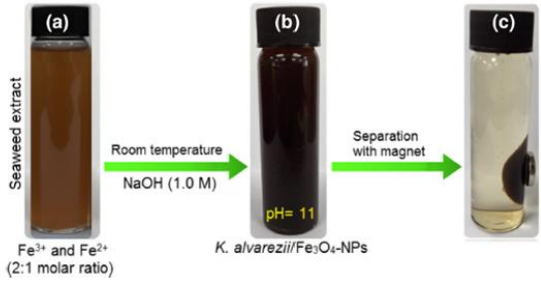

Figure 3: Procedure of plant extracted base green synthesis(Yew et al., 2016).

Shameli et al. obtained magnetite nanoparticles by using seaweed (K. alvarezii) extract. Figure 3 summarizes the applied process. Seaweed extract was obtained by keeping the dry plant in water for 24 hours without applying any heat treatment. The formation of magnetic nanoparticles was observed by mixing the extract and iron salts at room temperature for about 24 hours. The nanoparticles obtained are spherical in shape with magnetic properties and an average size of 14 nm(Yew et al., 2016). Likewise, Stamatis et al synthesized hybrid zinc oxide–iron oxide (ZnOFe) magnetic nanoparticles using Olea europaea leaf extract (Fotiadou et al., 2021).

2.3.2 Microorganisms Based Synthesis

Microorganisms such as bacteria possess reducing molecules necessary for forming metal oxide nanoparticles. Therefore, they carry out the synthesis of nanoparticles in them by the metabolic procedure. This process is called biomineralization. In addition, the method is preferred in magnetic nanoparticle synthesis as it allows shape and size optimization(Rico Martínez, 2019).In synthesis based on microorganisms, metal ions are reduced by the enzymatic system (NADH reductase).

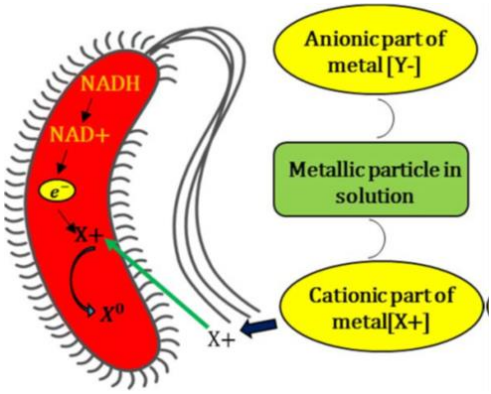

Figure 4: Representation of magnetic nanoparticle synthesis based on the use of bacteria(Rico Martínez, 2019)

In synthesis based on microorganisms, reduction of metal ions is carried out by the enzymatic system (NADH reductase) from intracellular and extracellular. A simple representation of the synthesis based on bacteria is given in Figure 4. Organic molecules other than enzymes and proteins in the synthesis medium serve as stabilizers(Rico Martínez, 2019), (Priya et al., 2021).

In another example, Tarafdar et al. prepared an iron nanoparticle with a size range of 10-24 nm using a fungal species, Aspergillus oryzae TFR9 (Tarafdar & Raliya, 2013).

3. Applications of Magnetic Nanoparticles

The unique magnetic properties, low cost and easy synthesis (especially the green synthesis option) of magnetic nanoparticles have made them attractive in the fields of technological and scientific applications. Synthesis in various geometric shapes with different surface properties and low toxic effects have allowed these particles to be used in a wide variety of application areas. These areas are the catalyst, data storage, energy, environment and wastewater treatment, agriculture, textile, food, defense technologies, biotechnology, and medicine etc., can be listed as(Ajinkya et al., 2020) . Although the application areas are so diverse, biomedical applications are the area where their use is most needed and where the studies are moving in that direction. This is because the magnetic properties of the particles can be used in imaging, thermal therapy, targeting, drug delivery, etc., making such processes more efficient(Ali et al.,

2021).

Some examples and explanations of applications, especially biomedical applications, are briefly explained below.

3.1 Magnetic Targeting and Drug Delivery

Drug delivery and targeting studies are of critical importance in the diagnosis and treatment of cancer. Magnetic particles are a vital candidate for these applications because of their magnetic properties. The drug or biomolecule is modified on the magnetic nanoparticle and directed to the tumor site with the magnetic field. Some of the molecules used as drugs in the literature in this way are: doxorubicin, epirubicin, paclitaxel/docetaxel, methotrexate .(Lyer et al., 2015).

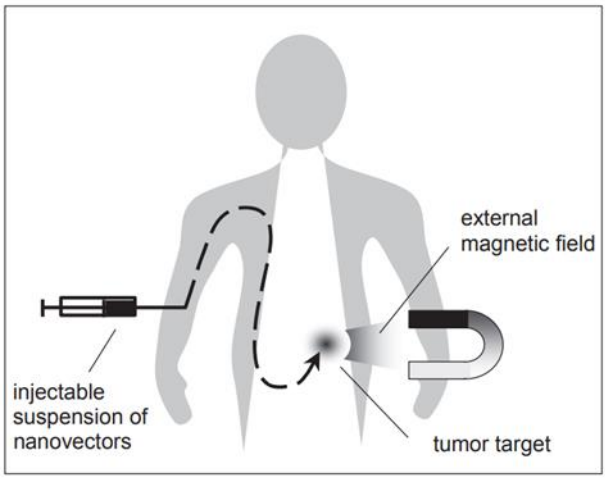

Figure 5: Schema magnetic drug targeting(Marchais & Hervé, 2007)

With magnetic drug targeting, since the drug is directed to the cancerous area with a magnetic force from outside the body, the toxic effects that chemical targeting agents may cause are reduced. The drug concentrated on the cancer tissue increases the effectiveness of the treatment(Tartaj et al., 2003).

3.2 Diagnosis and Imaging

Imaging methods are critically important for the diagnosis of defects. Magnetic Resonance Imaging (MRI) technique, which is one of these methods,

plays an important role, especially in diagnosing cancer, because of its sensitivity and not being harmful to the body. With the advances in MRI management, images close to microscopic resolution can now be obtained. With the use of magnetic nanoparticles as a contrast agent, the resolution of the MRI method will be further increased. As the magnetic nanoparticles agglomerate by targeting tumor regions, T1-T2 provides high contrast, and cancerous tissue and healthy tissue border are determined precisely and accurately(Ali et al., 2021), (Ajinkya et al., 2020).

3.3 Magnetic Hyperthemia

Killing unhealthy cells with high heat (hyperthermia) has been used in cancer treatment in recent years. Cancer treatment with hyperthermia is based on the principle that cancer cells die at temperatures above 42˚C, while healthy cells continue to exist at this temperature. The most critical shortcoming of the currently applied methods is that they are only effective in tumors close to the skin surface due to the heating provided by the beam. To kill the tumor cells in the inner regions, it is necessary to provide heating in the areas. This difficulty will be overcome with the magnetic hyperthermia method. Magnetic nanoparticles collected by magnetic targeting at the tumor site release heat when an alternating magnetic field is applied from the outside. When it exceeds 42˚C, healthy cells continue to exist, while tumor cells die. The heat release of magnetic nanoparticles under an alternating magnetic field is explained by Neel-relaxation, Brown-relaxation, and hysteresis loss mechanisms. Magnetic hyperthermia treatment will be used frequently in the coming years because of its very low side effects and effective results when combined with other drug treatments(Ali et al., 2021),(Tartaj,et al., 2003) , (Lu et al., 2007).

3.4 Other Magnetic Applications

In the coming years, it is aimed to use magnetic nanoparticles to combat environmental pollution. By using magnetic nanoparticles, a magnetic separation method of magnetic nanoparticles is provided to clean water and soil from heavy metals, organic chemicals, pesticides, dyes, and industrial wastes. Magnetic nanoparticles modified with capping agents attach to the polluting molecules from the medium and remove them from the polluted medium with the help of a magnet(Ali et al., 2021). The use of magnetic nanoparticles as

catalysts has become very attractive because they are easily separated from the medium by magnetic separation after the reaction is finished. It is challenging to separate other homogeneous catalysts from the reaction medium. Thanks to the photocatalytic property of these nanoparticles, organic water pollutants undergo photodegradation with the activation of sunlight and the pollution is removed(Shukla et al., 2021). In addition, magnetic nanoparticles are very suitable for energy and data storage applications. The use of magnetic nanoparticles in the field of energy, solar energy, electrochemical energy storage studies, and materials developed for fast data storage has become widespread(Zhang et al., 2010)., (Lu et al., 2007).

4. Conclusion

The development of technology, the change in the profile of people's needs and the resulting environmental pollution have led to the emergence of innovative approaches. Nanotechnology has emerged as a product of this innovative thinking system. The use of magnetic nanoparticles, which are the products of nanotechnology, has become popular in many applications such as health, energy, agriculture, and the environment. However, the wastes and chemicals used in producing these nanoparticles with classical methods harm the environment and living healthily. In addition, it causes high costs in using energy and chemicals in this way. The usage green chemistry approach overcomes this challenge. By using biological materials, sustainability is ensured by giving zero harm to the environment after synthesis. As the sustainability approach is adopted worldwide, green chemistry methods will be developed in the production of not only magnetic nanoparticles but also all industrial.

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