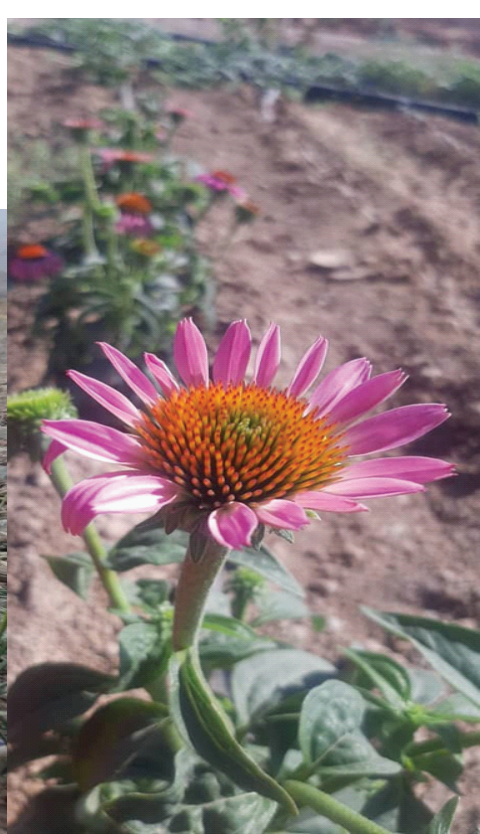




# NEW DEVELOPMENT ON MEDICINAL AND AROMATIC PLANTS-II

EDITOR

Assoc. Prof. Dr. Gülen ÖZYAZICI



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## **PREFACE**

Since ancient times, people have benefited from the plants around them for various purposes. Medicinal and aromatic plants have been used for centuries in the prevention, healing and protection of diseases. In addition, medicinal and aromatic plants are used in nutritional supplements, herbal teas, as spice, flavor and thickener and for functional food purposes.

Türkiye has an important place in the production and trade of medicinal and aromatic plants and dye plants thanks to its geographical location, climate and plant diversity, agricultural potential. According to the data of the Turkish Statistical Institute, in our country, about 20 kinds of medicinal and aromatic plants are cultivated on an area of 1.3 million decares.

The production of medicinal and aromatic plants in the last ten years has spread over a wide area compared to the 2000s. The production of many plants such as hops, thyme, anise, fennel, fenugreek, nigella sativa, black cumin, mint, rosemary, lavender, sage, caper, echinacea, chamomile, basil, coriander, nettle, lemon balm and basil continues. Currently, bulbous medicinal plants, wild thyme species, St. John's wort, laurel etc. the plants are collected from nature.

The quality and quantity of medicinal plants demanded by international markets a large number of projects have been completed and are still being carried out by the Ministry of Agriculture and Forestry, Development Agencies, universities, other institutions and organizations in order to ensure production.

In this book, information on the components of medicinal and aromatic plants, secondary metabolites, areas of use, new developments in agricultural and pharmacological properties of plants belonging to different genera and species are presented.

Assoc. Prof. Dr. Gülen ÖZYAZICI



## CHAPTER 1

### FACTORS AFFECTING THE PRODUCTION OF SECONDARY METABOLITES IN MEDICINAL PLANTS

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## **INTRODUCTION**

Plants are older than human life and are a source of food, air, medicine, and human treatment. Among these, medicinal plants as plant genetic resources and treasures have a major role in the composition of plant communities due to their high ecological flexibility in diverse climates. Such crops have the greatest wealth for any country and make a great contribution to the prosperity of the agricultural sector. For this reason, they are known as "green gold" in the scientific community. Also, medicinal plants have a special value in the biological, medical, and veterinary sciences in terms of the prevention and treatment of diseases. In recent years, the use of these plants has been cheap due to their effectiveness and has no side effects. People's interest and attention to the therapeutic effects of medicinal plants and their derivatives has led to a thriving trade in plants and more attention to the agricultural sector (Solidum, 2014).

A report released by the World Health Organization (WHO) shows that more than 80% of the world's population, especially in developing countries, uses medicinal plants and rich reserves of traditional medicine to meet their health needs. Also, the production and consumption of medicinal plants in the world have increased from \$ 14 billion in 2002 to \$ 124 billion in 2017, and according to the World Health Organization, it will reach \$ 5,000 billion in 2050. Fortunately, over the past decades, societies' views of medicinal plants and their healing effects have changed completely, and in a way, the re-approach

of industrial societies to medicinal plants and herbal medicines can be seen clearly.

Medicinal plants are collected from nature by nature or planted on farms and can be used as an important source of medicine by humans and even animals. These plants, as dynamic and large sources of income, have a very high potential for creating a prosperous economy and income from the gross national product (GDP). Their use in the pharmaceutical, food, and cosmetic industries and other related products, in addition to the beneficial effects on the development of the culture of consumption of medicinal plants and herbal medicines and their promotion, can serve as a potential means for generating foreign exchange earnings and reducing imports. At present, many countries in Western Europe and Eastern Asia, including China and India, are aware of this issue and, with the high production of these plants and through advances in industries related to traditional and complementary medicine, have attracted huge incomes and play a major role in their country's economy (Batugal, 2004; Anonymous, 2015).

Medicinal plants and natural substances and the active ingredients in them have long played a special and valuable role in traditional and natural medicine. There are 7500 to 8000 plant species in Iran, of which more than 200 species have medicinal and economic value. The growing population of Iran and also the growing need of the pharmaceutical industry for medicinal plants as a raw material for drug production show the importance and high status of these plants. Iran, with a brilliant history in medicine and potential, geographical

potential, climatic diversity, and having about 123 exclusive and endemic plant species and a great variety of native species, it should be able to use its high talents and capacities for economic development and growth by developing the cultivation, processing, and industry of Iranian medicinal plants and using itself as a lever. Major in the development of agriculture, processing, and industry of medicinal plants in the world, and has become one of the poles of the world's medicinal plant economy.

Increasing demand for medicinal plants, ease of collection from nature, and the low cost of this type of harvest, have accelerated the process of depletion of natural resources. Therefore, at present, the cultivation of medicinal plants and the improvement of important and valuable medicinal varieties, given the economic and health importance of these plants and their special place in global markets, should be at the forefront of the country's agricultural and industrial programs. In particular, medicinal plants can account for a significant share of non-oil exports and boost national economic output.

## **1. SECONDARY METABOLITES IN MEDICINAL PLANTS**

Medicinal plants are economical plants used by humans that store special and useful biochemicals in very small amounts, so the cultivation of medicinal plants is very common around the world. Today, the consumption of medicinal plants is increasing due to the side effects of chemical drugs. The active ingredients of medicinal plants



have a direct or indirect therapeutic effect and are used as medicine. The study of secondary chemicals began with the chemical decomposition of medicinal plants in the nineteenth century. The results of these studies, from the very beginning, showed that medicinal plants, in addition to general and basic compounds, each have at least one specific active ingredient.

Plant cells produce two classes of compounds, one is primary metabolites and the other is secondary metabolites. Primary metabolites are directly involved in growth and metabolism and include carbohydrates, lipids, proteins, and nucleic acids. In plants, primary metabolites are produced during the process of photosynthesis and then play a role in the formation of cell compounds. These compounds are produced in large quantities and with low economic value and are mainly used as raw materials for industry, food, and additives. Vegetable oils, fatty acids (for making soaps and detergents), and carbohydrates such as sucrose, starch, pectin, and cellulose are examples of primary metabolites (Ghaffari and Tavassoliyan, 2015).

Secondary metabolites, derived from the biosynthesis of primary metabolites and considered byproducts of primary metabolism, are complex molecules with low molecular weight, and their structure is different from that of the primary metabolites that are essential for cell survival (Adams, 2007). Also, these compounds do not participate directly in the developmental or reproductive stages of a living organism, and their role in growth, photosynthesis, crop conversion, or other major functions has not yet been discovered (Anonymous, 2011).

Secondary metabolites are mainly produced in certain species and families of the plant series. These compounds are stored in small amounts in the cell and are mainly produced in specialized cells at a certain stage of the plant's life cycle.

Also, these compounds do not participate directly in the developmental or reproductive stages of a living organism, and their role in growth, photosynthesis, crop conversion, or other major functions has not yet been discovered (Anonymous, 2011). Secondary metabolites are mainly produced in certain species and families of the plant series. These compounds are stored in small amounts in the cell and are mainly produced in specialized cells at a certain stage of the plant's life cycle, making them difficult to extract and purify compared to the primary metabolites produced in all cells.

In contrast to primary metabolites, such as carbohydrates, proteins, and nucleic acids, the absence of secondary metabolites in a plant does not lead to its immediate death, but may in the long run impair plant survival, fertility, or appearance. It may not cause any noticeable change. Undoubtedly, herbs are the most important and best source of secondary metabolites that have been known as valuable sources for generations. Secondary metabolites are widely used in various industries such as food, industrial, pharmaceutical, and health and are used in the manufacture of medicine, soap, essential oils, dyes, gums, resins, rubber, food seasonings, and beverages, etc. These compounds also have important functions in the plant itself, such as the function of hormones and growth regulators, microbial contamination,

absorption of pollen insects (especially anthocyanin pigment and terpenoid essential oil), as well as repelling herbivores and insects, which in turn causes Reduce animal and insect damage and aid in the survival of productive plants in their ecosystem (Wink, 2003; Karuppusamy, 2009; Summer et al., 2014).

## **2. FACTORS AFFECTING THE PRODUCTION OF SECONDARY METABOLITES IN MEDICINAL PLANTS**

Many factors in nature prevent the uniform and continuous production of secondary metabolites. These factors include physiological variables (organ development stage, pollinator activity period, plant organ, secretory structure, season diversity, mechanical and chemical damage), environmental conditions (climate, pollution, diseases and pests, soil factors), geographical diversity (longitude, altitude), genetic and evolutionary factors (evolution, genetic structure, mutation), storage and storage (storage time, temperature, humidity, air conditioning), which are among the factors affecting quantity, and the quality of the metabolites are secondary.

### **2.1. Physiological Variables**

#### **2.1.1. Organ development stage**

The growth stage of plant organs (leaves, flowers, and fruits) can be a determining factor in the composition of active ingredients (Ahmed et al., 2001; Badalamenti, 2004). In many cases, the yield of volatile compounds extracted from young flowers was higher than that of mature flowers. Continuously, the constituent compounds in different

vegetative stages of the plant show significant changes. Some of these compounds have small amounts of up to 10% in the early stages of flowering, which reach 50 to 70% in the full flowering stage. In some cases, significant amounts of volatile compounds accumulate before the organ is fully grown. For example, in basil (*Ocimum sanctum*), the amounts of eugenol and methyleugenol decrease with leaf development, which is due to the use of these compounds in lignin synthesis and increased oxidation of phenolic compounds by increasing the activity of polyphenol oxidase and peroxidase enzymes (Dey and Choudhuri, 1983). According to Manez et al. (1991), changes in the composition of adult plant volatiles are directly related to the chain and recycling of these compounds. In 2001, Pala-Paul et al. (2001) investigated the effects of growth stages on the essential oil content of *Santolina rosmarinifolia* in Spain. The results of this study showed that the amount of essential oil has an upward trend during the vegetative and flowering stages. Hodaib et al. (2002) also examined quantitative and qualitative changes in the essential oil of thyme (*Thymus vulgaris*) during the growing season and obtained the best essential oil yield at the beginning of the early flowering stage of 1.2%.

The results of quantitative studies of *S. cordifolium* essential oil showed that the amount of essential oil increases during plant growth. So the percentage of essential oil based on the dry weight of the sample used in the pre-flowering, flowering, and fruit ripening stages was 0.3%, 0.4%, and 0.7%, respectively. The amount of essential oil in the

ripening stage of fruits was 42.9% and 57.2% higher than in the flowering stage and before flowering, respectively (Amiri et al., 2007).

Daily fluctuations in the composition and yield of essential oils of various plants have also been reported. The change in compounds with plant organ development is a metabolic change in which, for example, the amount of monoterpenes decreases with changes in storage material, while in daily changes the change and conversion of terpenes depend on photosynthesis. Studies of mint and sage have shown that the catabolism of terpenes leads to the formation of glycoside derivatives that are transported to the roots and used to synthesize lipids (Croteau, 1987). Conversion of secondary metabolites to glycoside form is an efficient detoxification system to prevent toxicity due to the accumulation of secondary metabolites, which results in the production of water-soluble aglycone, which is widely used in pharmacy and produces an inactive product (Belhattab et al., 2005). It should be noted that some of the differences in the function and composition of active ingredients with limb development can be explained by the diversity of secretory structures. Plants with external secretory structures can release their leachate when the organ arrives due to cuticle destruction, but plants with internal leachate structures often have a stable function and a combination of secondary metabolites. Many studies have been performed on the quality of essential oil and its variation in the seeds of umbel species such as cumin (*Cuminum syminum*) (Moghaddam et al., 2005b), black cumin (*Carum carvi*) (Bouwmeester et al., 1995). Coriander (*Coriandrum*



*sativum*) (Msaada et al., 2007) and fennel (*Foeniculum vulgare*) (Telci et al., 2009) indicate that the percentage of essential oil in the early stages of growth is higher than in the mature stage and with seed development. Until full maturity, its content is reduced. Essential oils in umbrella plants accumulate in ducts called "vitae". These structures are present in large numbers in the early stages of seed development, which leads to a high amount of essential oil in seeds at this stage of development. The size of the essential oil ducts gradually decreases with grain ripeness, which leads to a decrease in the essential oil content of the grain at full ripeness (Bernath et al., 2001).

### **2.1.2. Pollinator activity cycle**

Many flowers pollinated by insects are fragrant. In addition to the appearance features that are attractive to pollinators (color and shape), the diffused fragrances of flowers are the most important signal for attracting and orienting pollinators, especially insects at night. Based on the type of odor released, insects can distinguish flowers from each other and show behavioral reactions necessary for pollination. Orchids have a proprietary system for producing pheromone-like compounds, 1.8 cineole constitutes 60% of the orchid fragrance, which is attractive to a large number of pollinating bees (Dodson et al., 1969). Methyl salicylate and methyl cinnamate attract a few bees. The aroma of plants, along with the amount of access to leachate or pollen maturation, are important factors that affect the activity of pollinators. In plants with daily pollinators, the release of volatile compounds reaches its maximum during the day, while in plants with nocturnal pollinators, a

strong odor is emitted from the plant between 19:30 and 7:30, with the highest amounts in the morning. Hours between 23:30 and 3:30 have been recorded (Ikeda et al., 1994).

### **2.1.3. Herbal materials**

In only a small number of plant species, the amount and composition of secondary metabolites extracted from different organs are the same and the composition of the active substance depends on the organ and tissue used (flowers, green tissues, skin, wood, fruit, seeds, roots) (Olawore et al., 2005). In a study, different organs (leaves, flowers, stems, and roots) of sage were studied in terms of the amount of essential oil and the variety of chemical compounds in them. The essential oil yield of different organs was 1.2, 0.6, 0.06, and 0.04% (w/w) for leaves, flowers, stems, and roots, respectively. In total, 44, 46, 42, and 45 compounds were identified in the essential oils of leaves, flowers, stems, and roots of this plant, which formed 99.6, 99.3, 98.2, and 99.4% of the total essential oil compounds, respectively. Cluster analysis of sage essential oil chemical compositions divided different organs into two main groups, in the first group, the leaf and stem organs were in one subgroup and the flower organs were in the other subgroup. In the second group, the root limb was placed. The obvious chemical diversity of the essential oils of this plant can be considered for the pharmaceutical and food industries as well as the expensive breeds of medicinal plants in the selection of each organ for consumption and breeding purposes (Hedayati et al., 2015). According to reports by Vojoodi et al. (2018), all shoots of Caspian yarrow (*Achillea filipendula*

Lam.) contained essential oil separately, but in terms of yield and constituents of essential oil, differences and similarities were observed.

#### **2.1.4. Structure of secretory organs**

The difference in the composition of essential oils from different parts of the plant can be attributed in part to the existence of distinct secretory structures that are unevenly distributed throughout the various organs of the plant. Plant volatile compounds are produced in differentiated secretory structures, which reduce the risk of toxicity and, on the other hand, provide high amounts of metabolic compounds in parts of the plant. Due to their defense and absorption roles, these compounds can be vital to the plant. The type and location of these structures are characteristics of different plant families. In some plants, the presence of more than one type of secretory structure has been reported. For example, in yarrow, there are two types of secretory structures: fluff and secretory ducts. These secretory structures do not always develop simultaneously, usually do not exude the same composition, and may have different modes of exudation. In addition, in a genus with internal secretory structures, the chemical composition of the secreted material may be similar, but the position of the secretory structure determines the role of these substances as a defense factor for the plant or an insect repellent, which is the case in some plants of the family Euphorbiaceae, as has been reported.

Morphological studies in plants have shown that the distribution of secretory and apical secretory hairs on the surface of the limb is also varied. In addition, the density of secretory hairs in vegetative and

reproductive organs is different from each other. In the milkweed plant (*Lenontis leonurus*), there are abundant shield hairs on both leaf and flower surfaces, while apical hairs are found only on the leaves and are very rare in flowers. These two types of fluff are also different in terms of the type of secretion. In shield hairs, secretions accumulate in the space below the cuticle until the cuticle is destroyed by an external agent and the material leaks out, while the apical hairs may release the secreted material through micropores (Konarska, 2017). In plants belonging to a species, the secretions from secretory glands may be similar to each other, such as sage, or different from each other, such as sunflower. In *Plectranthus* plants, the most important component of the essential oil is a diterpene, which can be seen as orange or reddish crystals after separation. The accumulation of this compound is limited to shield hairs, which are found in this plant only in the areas between the veins. Due to the accumulation of this substance, the hairs are orange or red, which can be distinguished from other hairs as a morphological sign (Figueiredo et al., 2008).

### **2.1.5. Variety of seasons**

In some species, the composition of the essential oil or active ingredient varies at different times of the year, so harvest time is important from an agricultural and economic point of view. In yarrow, Sesquia terpene hydrocarbons are the predominant compound during the growing season. On the other hand, hydrocarbon monoterpenes are the most important active ingredient during the yarrow flowering period (Figueiredo et al., 1992). There are daily changes in the quality of

secondary metabolites that may be related to pollinators, in addition to monthly and annual fluctuations or changes in the composition of the active ingredient of plants during the vegetative to reproductive stages. Variation in yield and composition of essential oils can be related to vegetarianism, climatic parameters (day length, temperature, and humidity), and the attack of fungal pathogens, especially during rainy months (Figueiredo et al., 1997). Harvest time depends on the specific plant species and is determined commercially based on the desired quality and quantity. For example, it is very important to determine the right time to harvest the vanilla plant. Vanilla seeds are usually harvested when they are green and have no flavor, and they are cured for three to six months. This treatment aims to make available the precursors affecting the vanilla flavor in the embryonic tissue around the seeds to the related enzymes that are often present in the outer wall of the fruit. These enzymes activate the interactions that hydrolyze the precursors to release effective compounds in the vanilla flavor, especially vanillin. If vanilla beans are harvested earlier, they are prone to mold and the amount of vanillin will be reduced, and if harvested in the post-ripening stage, the seeds will be cracked and of low quality, which will reduce their economic value (Hawkin et al., 2005).

#### **2.1.6. Mechanical and chemical damage**

The release of volatile compounds not only acts as a stimulant and adsorbent for plants, but also directly or indirectly plays a defensive role in plants. However, the effects of mechanical and chemical damage from wounding attacks, pest infestations, and herbicide treatments on



the yield and essential oil composition of medicinal plants have been less studied. Under normal circumstances, plants produce a number of secondary metabolites that are considered to be constructive. When they are exposed to any kind of damage, new production may take place. That is, compounds are synthesized in the plant that did not exist under normal conditions. This is called induction production. The difference between constructive and inductive production may not be very clear, as most aromatic compounds, usually produced by healthy plants, are induced after each injury. In most cases, these compounds are produced in larger quantities or in different proportions. The induced response is not only a function of plant species, but also depends on the developmental stage and the degree of access to water and light (Figueiredo et al., 2008).

The effect of damage on the quantity and quality of secondary metabolites of resin plants is of particular importance. In these plants, the resin accumulates in internal secretory structures such as cavities and ducts. In a species of pine (*Pinus pinaster*), creating a hole in the trunk increases the essential oil of the plant by 2.5, and inoculation with the mycelium of the fungus increases the yield of the essential oil by 60 times. The effect of adding mycelium on increasing the amount of terpenes is maintained longer than the effect of the wound. Wound and mycelial inoculation, on the other hand, do not affect the percentage of essential oil constituents. Foliar use of herbicides and growth regulators in sage has shown significant changes in yield and plant essential oil composition, although no correlation can be made between the results

(Figueiredo et al., 1997). Conifers produce resins of high ecological and commercial importance. Mono and diterpenes are found in nearly identical amounts, as are small amounts of sesquiterpenes in coniferous resin. In these plants, the resin accumulates in specialized secretory structures that may be simple spindle-shaped or very complex three-dimensional structures. After mechanical damage, insect feeding, or fungal attack, the resin accumulates in the axial ducts of the trunk skin and induces resin cavities, which are associated with the development of woody tissue. Martin et al. (2002) examined the morphological traits of cells after treatment with methyl jasmonate. Adjacent adrenal cells found a denser cytoplasm and a thinner cell wall, which allowed new resin cavities in addition to existing structural cavities. At 15 days after treatment, the lumen of the induced secretory ducts was fully visible, which resulted in the formation of a ring in the younger part of the wood tissue. At this time, the lumen began to fill with resin. In addition to inducing the formation of new channels, methyl jasmonate treatment increased the total accumulation of mono- and diterpenes while remaining unchanged for sesquiterpenes. Plants under stress or disease can produce high amounts of volatile compounds, which can be problematic and lead to allergic reactions in people who come into contact with such plants.

## **2.2. Environmental Conditions**

### **2.2.1. Weather**

Despite all the technological advances in agriculture, one of the factors beyond human control is climatic conditions. The production of secondary metabolites is highly dependent on climatic conditions. Storms, tornadoes, floods, or drought are some of the environmental conditions that can affect the condition of medicinal plants to a greater or lesser extent. Water stress seems to be directly associated with the increased production of volatile compounds in several plant species, including dill, tarragon, and basil. In coriander and thyme, yield increases are achieved under optimal irrigation conditions. Drought stress can limit photosynthesis in plants and alter nutrient uptake, carbon and amino acid content, and inorganic iron flow. In addition, a plant under stress is more vulnerable to attack by pathogens and vegetarians due to unfavorable weather conditions or limited nutrient uptake. A significant decrease in the production of volatile compounds in plants is observed during the months with the lowest temperatures and the lowest hours of sunlight (Figueiredo et al., 2008).

### **2.2.2. Infection**

It is difficult to identify the harmful effects of air pollution on secondary metabolites, especially their constituents, because the results are ambiguous compared to the response of plants to other factors. In addition, the effects of pollutants such as smoke coming out of vehicles and fire are increased by wind, rain, and temperature. Few studies have been conducted on the effect of air pollutants on the composition of the

active ingredients of plants, and sometimes these results are contradictory and vary according to the type of pollutant. A comparison of the yield and composition of essential oil in *Cistus albicans* grown in two areas of rural farms and around stone mines has shown that plants grown in areas adjacent to the quarry have more essential oil yield without changes in their chemical composition. Dust around roads, cement plants, and quarries is closing the holes and reducing the flow of carbon dioxide, which can change the quantity and quality of the production of secondary metabolites (Figueiredo et al., 2008).

### **2.2.3. Diseases and pests**

Many types of diseases and pests (mold, rust, black spot) can have detrimental effects on the shelf life of plant materials. In some cases, the introduction of a new product in places other than its native place causes the spread of diseases and pests. Up to 40% of the *Piper nigrum* black pepper crop may be destroyed by a pepper beetle attack. Infection of the hyssop plant with this virus reduces the yield of essential oil by 88% and changes the relative amounts of the constituents of the essential oil (Figueiredo et al., 2008).

### **2.2.4. Soil factors**

Many researchers believe that the physical, chemical and nutrient properties of soil are one of the determining factors in the composition of secondary metabolites, especially volatile compounds, which may be one of the factors that make a difference in the composition of the active ingredient of plants belonging to a particular species. Among soil-related factors, the role of nutrients is more important, because the

amount of these elements can be easily changed, and through this, significant changes can be made in the quantity and quality of medicinal plants. Due to the development of methods for extracting and measuring the active ingredients of medicinal plants and existing advanced techniques, several studies have focused on the effect of fertilizing different elements on the production of organs used by medicinal plants and secondary metabolites. Nutrients are not only effective in increasing the yield of medicinal plants like other plants, but also change the quality of the product. In addition, antagonistic or synergistic properties between nutrients lead to changes in the absorption of some elements necessary for the plant. In some cases, the presence of these elements in the essential oil is toxic. Therefore, fertilizer recommendations for medicinal plants should be taken into account as a fertilizer treatment may increase yields while reducing the number of active ingredients or changes in the composition of the essential oil that are not beneficial. Different nutrient treatments often indirectly affect the quantity and quality of secondary metabolites, although in some cases, nitrogen nutrition directly affects the amount and type of alkaloids (Kizil et al., 2017). Although the different properties of the soil affect how it grows and also the amount of active ingredients in plants, it is not possible to be satisfied with only the physical or chemical properties of soil in this regard. Planting and propagating a plant species in similar soils may lead to different results in terms of drugs or even how they grow. Because the uptake and metabolism of plants may be affected by other environmental factors, increasing the amount of nitrogen indicates an increase in the growth of

the tattoo plant, and only moderate amounts of nitrogen can bring the amount of alkaloids produced by this plant to the desired level. According to Hornok (1988), the use of three important nutrients (nitrogen, phosphorus, and potassium) increases the yield of essential oils from different plants, although the separate addition of these nutrients shows different results in terms of yield and composition of essential oils. Some sources indicate that the composition of the essential oil, contrary to its function, is not affected by the concentration of soil nitrogen (Figueiredo et al., 1997).

Many plant species' growth and survival are threatened in poorly drained soils, which reduces yields, and essential oil yields, and changes the composition of plant active ingredients. Low levels of calcium cause acidic conditions in the soil and plant growth in these conditions is reduced. Dense cultivation of plants due to allopathic effects and shading may have negative effects on the quantity and quality of crop production. Therefore, in the cultivation of any medicinal plant, the physical and chemical properties of the soil and the availability of nutrients should be taken into account (Figueiredo et al., 2008).

### **2.3. Geographical Diversity**

In many plant species, the effect of geographical location on the diversity of function and chemical composition of secondary metabolites has been proven, which leads to the identification of different chemotypes in a species. Differences in the composition of essential oils of plants belonging to species that have grown in different regions indicate the different environmental conditions of each region

(altitude, sunlight, type of precipitation, rainfall, and temperature). Considering the characteristics of the place of growth and the position of the plant in nature, one of the main factors that can have a great impact on the amount of essential oils and active ingredients in plants, The results of various studies indicate the relationship between habitat conditions and the chemical composition of different plants, and a high correlation between the geographical origin of plants and their effective compounds has been reported (Bertome et al., 2007).

Moghaddam and Farhadi (2015) have shown that the highest percentage of essential oil and sulfur accumulation in *Angula* (*Ferula assa-foetida*) is related to the average annual temperature and altitude of this plant and that with increasing altitude and decreasing temperature, the amount of this The parameters are reduced, but the study of existing chemotypes has shown that environmental factors do not affect the phytochemical composition of Angoze essential oil. In general, environmental factors affect the quantity and quality of secondary metabolites in three ways: 1) effect on the total amount of active ingredients of medicinal plants, 2) effect on the constituents of active ingredients, and 3) effect on the amount of plant dry weight production.

It should be noted that all these factors are related to each other, and the differences observed in plants of different regions can also be due to genetic differences. In addition, post-harvest processes and extraction methods can affect changes in the quantity and quality of medicinal plants.

## 2.4. Genetic Factors and Evolution

Genetic studies and hybridization of medicinal plants show that the composition of plant essential oils is controlled by genetic factors. Analysis of essential oils of many mint species shows that CC and Cc genotypes have  $\alpha$ -Terpineol to Limonene conversion system, and Carvone is obtained by oxidation of Limonene. The cc genotype produces a Menthadiene that becomes Pulegone and Menthol. In the species of yarrow, cypress, pine, sage, and thyme, the composition of the essential oil is genetically determined (Nemeth, 2005). Pirmoradi et al. (2013) reported the use of essential oil constituents in the separation of basil varieties from each other, which indicates the effect of genetic factors on the essential oil constituents of this plant.

Natural selection determines the change in the production of effective plant compounds. *Pandrosa*, for example, is rich in the aromatic compounds terpene  $\alpha$ -Pinene, myrcene and limonene. Investigation of changes in oleoresine constituents shows that natural selection is to preserve trees with high concentrations of limonene (Manez et al., 1991). Mechanisms that alter the formation of aromatic compounds in plants include 1) gene replication followed by divergence, in which the main activity of the enzyme is preserved, while a new function is obtained from the duplicate gene. 2) convergent evolution, in which new functions emerge independently several times. 3) The evolution of an existing gene without replication, in which the new enzyme's role is attained by losing the previous role. 4). Inhibition of enzymatic activity is caused by several factors, such as hybrid



formation, mutations, or chromosome rearrangement (Gang, 2005). In each case, they have seized it, despite obstacles we can scarcely imagine. In addition, a variety of enzyme functions can occur with very small changes in the structure of the enzyme. When enzymes are exposed to different constitutive or inductive environmental conditions, greater variability in enzyme activity occurs. If the production of new metabolites gives the plant superior adaptation, the production of this metabolite will be maintained or increased. Changes in protein expression do not necessarily lead to the cessation of enzymatic activity, but in some cases, may lead to the production of secondary metabolites in cells, tissues, or other organs (Figueiredo et al., 2008).

## **2.5. Maintenance**

The amount of secondary metabolites can be affected by the storage method. Drying of plant materials has negative effects along with physical and chemical changes that affect the appearance and aroma of the plant and may cause changes in the composition of the active ingredients. However, drying medicinal plants reduces the growth of microorganisms and prevents harmful biochemical reactions.

Light, humidity, temperature, plant age, pollution, and oxidation affect the yield and composition of active ingredients. Of course, in these cases, the type of secretory structures is also important. The volatile compounds in the internal secretory structures change less, while the external secretory structures (hairs) naturally release their secretions by destroying the cuticle. Therefore, they are more sensitive to mechanical damage caused by harvesting, transporting, and

packaging. The function and chemical composition of the active ingredients of plants such as dill, cumin, chamomile, basil, and ginger change drastically during storage and drying. In some other species, storage and drying do not affect the quantity and quality of volatile compounds.

The methods of preparing the plant after harvest are precisely defined and the principles that have been obtained as a result of numerous experiments and measurements of the amount of active ingredients in plants are the methods of harvesting or collecting, drying, crushing, grinding, sieving, including threshing and even harvesting, drying, crushing, grinding, sieving, threshing and even fermentation. These methods are different for maintaining and maintaining the active ingredients in different plant species. In some plant species, post-harvest changes are needed to develop the aroma, as in the case of vanilla. In vanilla, the hydrolyzing enzymes (beta-glucosidase and other glucoside hydrolases) and the substrate (glucovaniline, the precursor to the vanilla fragrance) are separated by position. Contact between the enzyme and the substrate leads to the release of vanillin and the aromatization of vanilla (Figueiredo et al., 2008).

In general, a medicinal plant's crop is economically viable when the amount of its secondary metabolites reaches a certain level. Ecological and genetic factors have effects on the growth, new, and biosynthesis of primary and secondary plant compounds. Although these compounds are made by guiding genetic processes, their construction is significantly influenced by environmental factors.

Because the environment is the most important factor affecting the expression of biosynthetic genes of secondary compounds in medicinal plants, therefore, recognizing the factors affecting the quality and quantity of active ingredients of medicinal plants has always been considered. The needs of the pharmaceutical industry for the active ingredients of plants are so great that it is not possible to obtain them from nature. Hence, many of these plants must be grown on large farms. Some factors, such as selecting the best species and using sufficient care in selecting the propagation method, reviewing and selecting the appropriate machinery for planting, harvesting, and drying them, are necessary to achieve the highest quantity and quality of active ingredients in medicinal plants (Figueiredo et al., 2008).

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## CHAPTER 2

### **LEGUME FORAGE CROPS WITH MEDICINAL VALUE AND THEIR SECONDARY METABOLITE CONTENTS: *Medicago sp., Onobrychis sp., Melilotus sp., and Lupinus sp.***

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## INTRODUCTION

Nowadays, when climate change and the risky life standard caused by it are increasing in parallel with the rapid growth of the world population, ensuring food security stands out as the biggest vital challenge. With their many beneficial properties, especially biological nitrogen fixation, legume plants play an important role in supporting both environmental sustainability and food security (Özyazıcı and Açıkbaş, 2021; Wijekoon et al., 2021). Legume forage crops in this group are rich in many essential nutrients, especially minerals, vitamins, fibers, and proteins, and they are also known as excellent sources of other beneficial compounds called secondary metabolites (Sabudak and Guler, 2009; Muzquiz et al., 2012; Butkutė et al., 2016). Due to these rich compounds, legume forage crops constitute an important component of roughage in animal production, and moreover, their plant parts are used as functional food supplements, and many species of some genera are used in the treatment of many diseases in traditional medicine. Nowadays, when some legume forage crops species are sold as powder, capsules, tablets, or seeds in health food stores (Wijekoon et al., 2021), it is important to know these secondary metabolites, which are concentrated in various plant parts of this group of plants and have an important potential for animal and human health, and their functions.

In this section, the secondary metabolites contained in some legume forage crop species, which have an extremely important value as a forage crop, were discussed, and it was attempted to examine the

effects of these components on roughage quality and animal and human health.

### **1. *Medicago* sp.**

The *Medicago* genus contains 83 different herbaceous or shrub plant species with high forage value belonging to the family Fabaceae (Heyn, 1963; Lesins and Lesins, 1979). The species in this genus are generally rich in protein, minerals, and vitamins, and their forage quality, nutritive value, and palatability are quite high. However, in addition to these properties, they also contain various secondary metabolites such as coumarins, isoflavones, naphthoquinones, alkaloids, and saponins (Barnes et al., 2002, 2007; Tava and Avato, 2006; Golawska et al., 2012). In this sense, saponins and flavonoids are the most interesting and well characterized among the secondary metabolite classes produced, especially in alfalfa (*Medicago sativa*) (Rafińska et al., 2017).

In recent years, attention has been drawn to the saponins contained in some plants used in animal and human nutrition, and it has been reported that such compounds have both beneficial and harmful properties. Tava and Avato (2006) reported that the *Medicago* genus represents a valuable source of saponins. In many studies, saponins were identified in other *Medicago* genera such as *M. sativa*, which is the most well-known and very valuable forage crop species in the *Medicago* genus (Massiot et al., 1988, 1992; Oleszek et al., 1990, 1992a, 1992b; Timbekova et al., 1996; Bialy et al., 1999; Huhman and Sumner, 2002), and *M. arabica* (Zbigniew et al., 2004), *M. arborea*

(Tava et al., 2005), *M. hybrida* (Bialy et al., 2006), *M. lupulina* (Oleszek et al., 1988), *M. polymorpha* (Kinjo et al., 1994) and *M. truncatula* (Huhman and Sumner, 2002; Kapusta et al., 2005), and the chemical profiles of these saponins were revealed. However, the function of saponins in *Medicago* plants has not been fully understood.

Nevertheless, saponins have come to the forefront with their antifungal and antimicrobial activities in *Medicago* species. In this sense, it was reported that saponins in *Medicago* plants were considered constitutive resistance factors involved in defense mechanisms, especially against pathogens (D'Addabbo et al., 2011). The studies have determined that saponins mainly isolated from *M. sativa* have antimicrobial activity against plant pathogens depending on their structure (Polacheck et al., 1986; Gruiz, 1996; Jurzysta and Waller, 1996; Avato et al., 2006). Indeed, a study conducted with *Medicago* species reported that the genera exhibited high activity against some Gram-positive bacteria (*Bacillus cereus*, *B. subtilis*, *Staphylococcus aureus*, and *Enterococcus faecalis*), and in this sense, especially *M. arabica* had a wider spectrum of action. The same study reported that different antifungal activity was observed against *Saccharomyces cerevisiae*, and while antimicrobial properties observed for *M. sativa* and *M. arborea* were associated with medicagenic acid, the bioactivity of *M. arabica* was due to the content of hederagenin (Avato et al., 2006). Saniewska et al. (2006) indicated that the saponins of *M. hybrida* had significant antifungal activity and that the roots of this plant could be a rich source of natural fungicides. In their in vitro study, D'Addabbo

et al. (2011) stated that saponins obtained from *M. sativa* could be good candidates for natural nematicide formulations. Abbruscato et al. (2014) reported that the saponin mixtures obtained from the tops and roots of alfalfa (*M. sativa* L.) had antifungal effects against *Pyricularia oryzae* in *Oryza sativa* L. ssp. *japonica* and that prosapogenins obtained especially from the tops were active compounds to prevent fungal attacks, and therefore, these substances could represent a promising and environmentally friendly treatment for controlling rice blast if they were properly formulated. D'Addabbo et al. (2020) reported that saponin-rich extracts and plant biomasses obtained from *M. heyniana*, *M. hybrida*, *M. lupulina*, *M. murex*, and *M. truncatula* could be quite suppressive against root-knot nematodes.

Furthermore, saponins, which are important secondary metabolites of biosynthetic origin included in the triterpenoid group of terpenes (Tiring et al., 2021), are also characterized by a number of pharmacological properties with antibacterial, antiviral and antitumor (Tava and Avato, 2006; Avato et al., 2017), and anti-inflammatory (Bhogireddy et al., 2013) activities. In their study, Li et al. (2020) reported that neuroprotective triterpenoid saponins extracted from *M. sativa* L. could provide bioactive substances for treating neurodegenerative diseases. Mansourzadeh et al. (2022) reported that *M. sativa* L., which had been traditionally used in the treatment of various disorders and in the human diet for at least 1500 years and was first cultivated in ancient Iran, might be useful in the treatment of diseases including diabetes as a herbal medicine.

Saponin-rich plant extracts are also used to manufacture cosmetic products such as lipstick, shampoo, and toothpaste (Tava and Avato, 2006).

Another biological activity of alfalfa saponins is insecticidal activity. In a study, partially purified saponin mixtures from alfalfa leaves showed insecticidal activity for *Lobesia botrana* and *Adoxophyes orana* (Tava and Odoardi, 1996). Another study on insects reported that the Colorado potato beetle (*Leptinotarsa decemlineata*) fed on potato leaves treated with 0.5% alfalfa saponin solution died within 4-6 days, and in this sense, alfalfa saponins showed strong deterrent activity (Szczepaniak et al., 2001). In addition to this information in the literature, there are studies supporting the insecticidal activities of alfalfa (*M. sativa*) saponins against various insects *Tenebrio molitor* (Shany et al., 1970), *Acyrtosiphon pisum* (Harris) (Pedersen et al., 1976), *Empoasca fabae* (Nozzolillo et al., 1997), and *Spodoptera littoralis* (Adel et al., 2000). The study conducted with alfalfa (*M. sativa* L.) saponins emphasized that the saponin composition in alfalfa changed with plant development, which might adversely affect the development of certain insect pests such as alfalfa aphid, suggesting a potential biological role of alfalfa saponins (Mazahery-Laghab et al., 2011).

Likewise, some other studies reported that *M. sativa* saponins showed fungicidal (Zehavi and Polacheck, 1996), anti-bacterial (Timbekova, 1995), and nematocidal (Argentieri et al., 2008; D'Addabbo et al., 2009) activities.

Furthermore, the allelopathic activities of saponins were also emphasized. It was reported that alfalfa produced allelopathic saponins, which might lead to significant reductions in the yield of crops grown after it (Gorski et al., 1991; Wyman-Simpson et al., 1991; Miller, 1992). These yield reductions were usually associated with old (aged) alfalfa (Mishutin and Naumova, 1955; Waller et al., 1993). Mishutin and Naumova (1955) indicated that the saponins leaking into the soil from the roots of alfalfa for 3-4 years were allelopathic agents and led to a reduction in the yield of the cotton plant planted immediately after alfalfa. Nevertheless, Wyman-Simpson et al. (1991) also emphasized that the amount, structure, type, and biological activity of saponins found in the roots of alfalfa varied over time. On the other hand, it was emphasized that there was a need for more studies to use alfalfa saponins as an effective weed control (Waller et al., 1993).

Lucerne (*M. sativa* L.) saponins are also known as important antinutritional factors that may hinder the nutritional value of lucerne species (Pecetti et al., 2006). In this sense, saponins reduce microbial fermentation and food digestion in ruminants and negatively affect microbial protein synthesis (Sen et al., 1998). Considering that different animal species have different sensitivity to saponins, there is no exact information for farm animals on lethal doses or minimum inhibition concentration of lucerne saponins (Pecetti et al., 2006). However, it was reported that poultry was more affected by lucerne saponins compared to other farm animals (Pecetti et al., 2006) and that a cultivar with a high saponin content led to more live weight reduction in chicks

(Pedersen et al., 1966; Pedersen and Wang, 1971). It is recommended to be careful while feeding animals with lucerne harvested at the end of summer and especially in aged meadows (Pecetti et al., 2006).

Saponins are found in freshly squeezed green alfalfa juice at a rate of approximately 2-3% (Rafińska et al., 2017), and the content and composition of saponins vary depending on the cultivar, environmental conditions, plant maturity stage, and plant parts (Tava and Pecetti, 1998; Pecetti et al., 2006; Golawska et al., 2012).

Another secondary metabolite found in alfalfa is flavonoids. Flavonoids generally protect the plant against bacteria, fungi, and insects (Pietta, 2000; Kelly et al., 2002). Rafińska et al. (2017) reported that alfalfa cultivars resistant to nematodes had high isoflavone levels.

Flavonoids also play a role in allelopathic interactions between plants (Rafińska et al., 2017). In this sense, it has been reported that formononetin and medicarpine, which are among some *M. sativa* isoflavones, inhibit germination in *Allium cepa* (Macias et al., 1999) and naringenin, one of the flavonoids, inhibits the root growth in *Glycine max* seedlings (Bido et al., 2010).

Raeeszadeh et al. (2022), who stated that *Medicago sativa* was a tonic plant rich in protein, vitamins, and minerals used in the treatment of many diseases due to its pharmacological properties, reported that alfalfa methanolic extract, whose total phenol and flavonoid level and free radical scavenging activity of its extract were 51.68 mg GAE/g, 18.55 mg QE/g, and 350.91 µg/ml, could recover nicotine-induced liver



injury in a dose-dependent manner by improving inflammation and oxidative damage. The researchers also stated that the high antioxidant power of alfalfa was probably due to the polyphenolic compounds it contained, these compounds captured free radicals and also reduced the stress and oxidative effects of nicotine.

In conclusion, *Medicago sativa*, which is present in the *Medicago* genus and is the most widely cultivated and known worldwide, is one of the plants rich in secondary metabolites characterized by various vitamins, especially vitamin C, as well as different types of compounds. With these antioxidant properties, alfalfa has been reported to have significant effects on some diseases such as diabetes, cancer, and kidney disorders. Therefore, *Medicago sativa* has been an important plant used for medical purposes around the world (Mirzaei et al., 2015).

Adult plants of black medic (*Medicago lupulina* L.), which is present in the *Medicago* genus and is an annual and/or short-lived perennial species native to Eurasia (Kicel and Olszewska, 2015), contain significant amounts of primary metabolites, including carbohydrates, soluble sugars, and proteins (Somme, 1968; Butkutė et al., 2017). Moreover, *M. lupulina* also contains significant amounts of various active phenolic compound groups such as coumestanes, isoflavonoids, and other flavonoids (Butkutė et al., 2017). With these contents, it can be said that *M. lupulina* has antioxidant, antiradical, and reducing activity properties. Indeed, *M. lupulina* leaf extract was reported to have antibacterial, antifungal, and antitumor effects (Baloch et al., 2013). In another study, *M. lupulina* extracted with glycerol was

reported to be a promising product that could be used for direct inclusion in antidiabetic food supplements and cosmetics (Jakupović et al., 2021).

The saponin contents of different plant parts of *M. lupulina*, which is a natural plant of rangeland areas in many parts of the world, were examined, their antioxidant activities were evaluated, and the biological significance of the presence of these compounds was discussed (Oleszek et al., 1988; Kicel and Olszewska, 2015).

*M. lupulina*, a traditional and medicinal plant, is a valuable source of new pharmaceutical agents (Eskandari and Doudi, 2016). A study reported that *M. lupulina* essential oil had a significant antileishmanial effect in preventing leishmaniasis disease caused by different species of *Leishmania* parasites. However, it was also emphasized that more experiments should be carried out on this subject (Eskandari and Doudi, 2016).

## **2. *Onobrychis* sp.**

Sainfoin included in the Fabaceae (Legumes) family spreads in the northern temperate regions of the world, and Iran and Türkiye are known as the center of diversity for this genus (Yildiz et al., 1999). There are two important agricultural types of it, including *Onobrychis sativa communis* 'Ahlefed' and *O. sativa bifera* 'Hort.' (Bhattarai et al., 2016). Nevertheless, sainfoin cultured on earth is *Onobrychis sativa* Lam. (= *Onobrychis viciifolia* Scop.), called common sainfoin (Manga et al., 2003). Very high-quality forage can be obtained from sainfoin, which is mowed during the flowering period, with high crude protein

content and low acid detergent fiber and neutral detergent fiber ratio. With this property, sainfoin has a forage value comparable to alfalfa and other legume forage crops as roughage (Manga et al., 2003; Bhattarai et al., 2016).

Moreover, sainfoin, which is a good source of flavonoids (Ahmad et al., 2010; Ince et al., 2012), especially its fresh part, has high nutritional value due to secondary compounds (polyphenols, carotenoids, tocopherols, and proanthocyanidins) with antioxidant properties (Mueller-Harvey et al., 2019; Rufino-Moya et al., 2019). In this sense, these main bioactive components in sainfoin are generally flavonoids and condensed tannins (CTs) (Quijada et al., 2015). As is known, flavonoids play an important role as antioxidant, antimicrobial, antihelminthic, and antiparasitic agents. Tanniferous forages have higher antiparasitic activity (Kommuru et al., 2014).

It has been reported that the fact that sainfoin reduces parasites in the digestive tract and improves the digestion of organic matter and protein in ruminants (Hoste et al., 2012) is due to the polyphenolic compounds (especially tannins) in its structure (Bhattarai et al., 2016). It has been indicated that these secondary metabolites in the leaves of sainfoin prevent gas formation in the rumen of ruminants and the bloating caused by it (Navasardyan et al., 2009; Wijekoon et al., 2021), have antimicrobial effects in the rumen (Hassanpour et al., 2011), increase the resistance of animals against various diseases and have anthelmintic and anticoccidial effects (Zhou and Doctor, 2003; Tikhvinsky et al., 2007; Navasardyan et al., 2009; Zaripova et al.,

2010). Some other researchers have also reported that CT in sainfoin helps reduce methane production in cattle, prevents bloating and improves meat quality (Acharya, 2015; Girard et al., 2016; Huyen et al., 2016). Marais et al. (2000), who emphasized that the threshold levels of CTs were important in addition to these positive effects of CTs in the sainfoin on animal welfare and health, observed that a higher CT level above the medium level (2-4% DM) formed complexes with proteins, which led to the inactivation of many digestive enzymes and reduced the digestibility of proteins. Therefore, the interaction of proteins and CT is important for the protection of forage proteins in the storage of forages, especially in the formation of high-quality silage forage and during rumen digestion (Huang et al., 2022). Huang et al. (2022) reported that CT in sainfoin significantly reduced the dry matter loss, lactic acid, acetic acid, and ammonia nitrogen content during ensiling and inhibited lactic acid bacterial fermentation by inhibiting fungal activity and reducing *Pediococcus* activity due to different strains. However, there are still not sufficient studies on this and similar antinutritional effects of sainfoin.

It can be said that the roles of sainfoin, which is the natural plant of the rangeland of the Eastern Anatolia Region in Türkiye and has a very high forage efficiency and quality, in increasing yield and quality in animals can be easily associated with the polyphenol compounds it contains, especially flavonoids. Indeed, Tufarelli et al. (2017) emphasized that flavonoids could improve the growth of animals and the quality of animal products.

In a study examining the parasitological status, antibody responses, and antioxidant parameters of the lambs experimentally infected with a gastrointestinal nematode (*Haemonchus contortus*) during the consumption of sainfoin pellets (SFPs) for 14 days, aqueous and ethanolic extracts of sainfoin pellets containing tannins, alkaloids, and flavonoids were used. The study reported that the treatment of lambs infected with *H. contortus* with SFPs directly affected the dynamics of infection in lambs and indirectly improved the resistance of animals against *H. contortus* by activating the antioxidant defense system and antibody response (Komáromyová et al., 2022). Another study stated that SFPs affected the composition of methanogenic bacteria in the rumen of lambs, reduced methane emissions, and thus, SFPs helped reduce the environmental burden of methane and minimize the negative effects on animal health. In the same study, the researchers indicated that the use of SFPs could be very beneficial within the scope of good agricultural practices (Petrič et al., 2022).

Due to these secondary compounds in sainfoin, it is also used in traditional medicine by boiling its green parts and roots (Waite, 1987). As a result of a study conducted with sainfoin (*O. viciifolia*), it was suggested that the acetone and methanol extracts of the sainfoin plant with high antioxidant properties could be used for pharmaceutical studies (Ince et al., 2012). Wijekoon et al. (2021) indicated that sainfoin (*O. viciifolia* Scop.) was a legume plant under development as forage crop in Canada with potential benefits to animal and human health and sainfoin-containing CTs gained popularity in western Canada due to its

benefits for cattle. The same researchers emphasized that CTs reported in sainfoin played a role in lowering blood pressure and in detoxification and providing anticancer properties in humans. Sainfoin is considered a model nutraceutical legume with its CTs content in Europe (Hoste et al., 2015). Nevertheless, despite the superior nutritional profiles and the presence of secondary metabolites, it was also indicated that no specific study was conducted on the human health benefits of sainfoin or its extracts (Wijekoon et al., 2021).

### **3. *Melilotus* sp.**

Sweet clover (*Melilotus spp.*), a forage crop genus belonging to the Leguminosae family, contains important species with high forage value. Yellow sweet clover [*Melilotus officinalis* (L.) Pallas] and white sweet clover (*Melilotus albus*) are the most widely grown important members of this genus (Darbyshire and Small, 2018). In addition to being used as forage, they are also commonly used as nectar plants, green manure, and soil conservation crops (Stickler and Johnson, 1959; Zhang et al., 2018; Mikhailova et al., 2022). These species are also recommended to be used in the phytoremediation of soils contaminated with heavy metals (Steliga and Kluk, 2021).

High coumarin content of the species *Melilotus albus* with good forage productivity (Rogers et al., 2008) and *Melilotus officinalis*, which is also considered an invasive species (Josifović et al., 1972) but can also be used as forage (Manga et al., 2003), limited their widespread use in roughage production (Nair et al., 2010; Luo et al., 2016) because this substance causes sweet clover bleeding disease in farm animals

(Kitchen et al., 2002; Nair et al., 2010). The compound mainly responsible for the disease is called dicoumarol, and some researchers argue that there is a correlation between high coumarin and dicoumarol content (Muir and Goplen, 1992; Kitchen et al., 2002). Nevertheless, *Melilotus albus* and *Melilotus officinalis* species have industrial and medicinal value due to their coumarin content (Pleșca-Manea et al., 2002; Cong et al., 2012; Mladenović et al., 2016; Zhang et al., 2018). In other words, *Melilotus* species are also a well-known medicinal plant used for coumarin extraction due to its relatively high coumarin content (Wu et al., 2021).

Coumarin, which is the parent compound of a large class of naturally occurring phenolic compounds (Maggi et al., 2011), is commonly used as a fragrance in industrial products together with its derivatives and has a medicinal value (Yarnell and Abascal, 2009; Luo et al., 2017). Coumarins have biological functions and/or pharmacological properties such as antioxidant (Bajerová et al., 2014), anti-inflammatory (Pleșca-Manea et al., 1999; Witaicenis et al., 2014), antibacterial (Céspedes et al., 2006), anticancer (Lata et al., 2015; Aras et al., 2016), anticoagulant (Lei et al., 2015; Sun et al., 2020), antiviral (Hassan et al., 2016) and termiticidal (Zhang et al., 2018). It is reported that coumarin has the effects of strengthening lymphatic vessels, stimulating blood and lymph flow, and therapeutic effects on chronic venous disease (CVD) (Perrin and Ramelet, 2011; Venugopala et al., 2013; Sowa et al., 2019). Furthermore, it has been stated that coumarins are also involved in processes such as defense against phytopathogens,

response to abiotic stresses, regulation of oxidative stress, and hormonal regulation (Bourgaud et al., 2006; Stringlis et al., 2019).

Coumarin is a plant secondary metabolite in *Melilotus* (Evans and Kearney, 2003), and the coumarin content in *Melilotus* species varies between 0.06 and 1.39% (Sarić, 1989; Pleşca-Manea et al., 2002; Nair et al., 2010; Luo et al., 2014). Coumarin obtained from *Melilotus* species is used as a component of anticoagulant drugs in the pharmaceutical industry (Casley-Smith et al., 1993). *Melilotus officinalis* extract was also reported to be used in treating diabetic foot ulcers (Chorepsima et al., 2013). In addition to its reported medical properties, melilot is also used to add flavor and aroma to foods and beverages (Ehlers et al., 1997). Moreover, it was reported that dried *Melilotus* species were also put in chests containing clothes and sheets to protect them from moths in Europe (Keating and O'Kennedy, 1997).

*Melilotus officinalis* also contains compounds such as melilotic acid and o-coumaric acid, melilotin, phenolic acids, steroids, saponins, fats, triterpenes, carbohydrates, sugar, anthraquinone glycosides, mucilage, choline, alcohols, uric acid, flavones, volatile oils, resins and tannins, as well as coumarin (Harnischfeger and Stolze, 1983; Pleşca-Manea et al., 2002; Al-Snafi, 2020). However, *Melilotus officinalis* is on the list of plants prohibited for use in food supplements (Nikolaev et al., 2019).

A study examining the allelopathic activity of aqueous extracts obtained from the leaves of *Melilotus officinalis* for seed germination and seedling growth in wheat reported that high concentrations (25-



30%) of the extracts significantly reduced the seed germination and root length of wheat seedlings (Siyar et al., 2017). This effect of coumarin, which inhibits the germination of plant seeds and seedling growth, was also stated in some other studies conducted with *Melilotus officinalis* (Wu et al., 2014, 2015).

In the study performed on 20 ecotypes of *Melilotus officinalis* collected from the northwestern pastures of Morocco, it was reported that the phenolic content of ecotypes varied significantly according to the maturation stage and origin of the ecotype and the total phenols, total tannins, and condensed tannins contents varied between 2.01-2.69%, 0.44-1.15%, and 0.04-0.14%, respectively, among ecotypes (Ayadi et al., 2021).

In 17 white sweet clover genotypes (*Melilotus alba* Desr.) collected from the natural flora of Bilecik province in Türkiye, it was determined that total phenolic, total flavonoid, radical scavenging activity (DPPH) and condensed tannin contents varied between 1.909-7.427 mg GA g<sup>-1</sup>, 9.900-25.621 mg QE g<sup>-1</sup>, 18.054-43.909%, and 0.372-0.886%, respectively (Yıldırım et al., 2021).

It was argued that the compounds released during the degradation of *Melilotus officinalis* suppressed weeds due to its allelopathic activity (Blackshaw et al., 2001). Yellow sweet clover can be used as a natural herbicide for weed control by intercropping, rotation, and soil mulching (Wu et al., 2010). In their study with the yellow sweet clover extract with coumarin content, constituting 1.152% of its dry matter, Wu et al. (2016) reported that a dose of 40 µg/mL coumarin significantly

inhibited the seed germination and seedling growth of *Lolium multiflorum*, *Polygonum aviculare*, *Trifolium pratense*, *Veronica persica*, *Poa pratensis*, *Chenopodium album*, and *Plantago asiatica* and completely inhibited the seed germination and seedling growth of *Lolium multiflorum*, *Trifolium pratense*, and *Polygonum aviculare* species. The same researchers indicated that coumarin with strong inhibition effects on seed germination and seedling growth in many weeds could be used as a natural herbicide. Mikhailova et al. (2022) reported that the toxic effect of *Melilotus officinalis* extracts was mainly due to the presence of coumarin, had high allelopathic activity, and as a result of laboratory and in situ studies, the aboveground part of *Melilotus officinalis* was a potential biopesticide source with a broad spectrum (bioherbicidal, insecticidal, and fungicidal) effect.

While the coumarin content in *Melilotus* varies according to the species, it also varies according to the variety of the same species and the cultural processes applied. In their study, Sowa-Borowiec et al. (2022) reported that sowing density did not affect the coumarin content of leaves, stems, and flowers of white sweet clover (*Melilotus albus*); however, the change of coumarin in leaves and stems according to the harvesting stages was significant. The researchers reported that the coumarin content of the leaves and stems decreased depending on the progress of the harvesting stages and that the amount of coumarin in the leaves and stems, which was 1.82 and 2.28 mg/g, respectively, before budding, was found to be 0.74 and 0.59 mg/g in the full flowering period, respectively.

#### **4. *Lupinus* sp.**

Lupin belongs to the genus *Lupinus* and the Fabaceae family, and its role in agriculture dates back to ancient times (Anonymous, 2013). There are currently more than 400 known species, and the types that are cultivated in the world among them are white lupin (*Lupinus albus* L.), blue lupin (*Lupinus angustifolius* L.), yellow lupin (*Lupinus luteus* L.), and pearl lupin (*Lupinus mutabilis* L.) (Pastor-Cavada et al., 2009). These species are also called sweet lupines due to their low alkaloid content and, therefore, less toxicity (Aydın, 2021). Lupins that are used for feed and silage forage, soil erosion control and green manuring (Manga et al., 2003; Visvanathan et al., 2020; Hussien, 2022) have also been used as a protein supplement in recent years, and especially blue lupin (*L. angustifolius* L.) is considered an alternative to soybean due to its high protein content (Garmidolova et al., 2022). Lupin grain is rich in protein (30-40%) and also has high dietary fiber such as soybean (28-30%), a low fat ratio (6%), and very little starch (Phan et al., 2007; Guemes-Vera et al., 2012). Lupin is also a good source of nutrients in terms of minerals and vitamins (Martínez-Villaluenga et al., 2006). Furthermore, white lupin has also created a growing market for human consumption due to the development of low alkaloid varieties that do not contain protease inhibitors (Hamama and Bhardwaj, 2004).

Nevertheless, lupins also contain different secondary metabolite groups. The most important secondary metabolites in the genus *Lupinus* are alkaloids found in high concentrations (>1 mg/kg dry weight) (Hama et al., 2022). Furthermore, lupins also contain phytochemicals

such as polyphenols, tannins, flavonoids, carotenoids, and phytosterols (Oomah et al., 2006; Kalogeropoulos et al., 2010; Boukid and Pasqualone, 2022).

Considering a positive relationship between total phenolic content and antioxidant activity (Siger et al., 2012), the antioxidant properties of lupin can be mentioned. Indeed, in a study, the antioxidant activity of lupin was associated with the presence of high concentrations of total phenolics and phospholipids in lupin extracts (Tsaliki et al., 1999). However, it should not be overlooked that other phytochemicals in lupin seeds will contribute to antioxidant activity.

Lupin alkaloids lead to acute toxicity in humans in the form of neurological, cardiovascular, and gastrointestinal disorders (Koleva et al., 2012). Although lupin alkaloids are toxic at high concentrations, attention has also been drawn to their antimutagenic, antibacterial, antifungal, and anticancer properties (Khan et al., 2015). Zielinska et al. (2008) and Martínez-Villaluenga et al. (2009) emphasized that lupin seeds were effective in reducing cancer due to their antioxidant content.

A study examining the antibacterial properties of *L. albus*, *L. luteus*, and *L. angustifolius* (Lampart-Szczapa et al., 2003) reported that the extracts obtained from the lupin hull exhibited antibacterial activity against indicator strains and the same effect was not observed in lupin cotyledons. The same study indicated that the extracts obtained from *L. albus* showed the strongest antibacterial activity, while *L. luteus* extracts showed the lowest antibacterial activity. Likewise, Erdemoglu

et al. (2007) indicated the antibacterial activity of alkaloid extract obtained from lupin seeds.

A study investigating the anti-inflammatory activity of the extract obtained from *L. mutabilis* seed in rats found that lupin extracts provided a significant reduction in edema formation and the anti-inflammatory effect of lupin extract was significantly higher compared to the control group (Gamarra-Castillo et al., 2006). Furthermore, it was reported that the lupin seed extract showed antifungal (Woldemichael and Wink, 2002; Erdemoglu et al., 2007; Dueñas et al., 2009) and antiviral (Barakat et al., 2010) activity. These antifungal properties of lupins are due to the phenolic compounds they contain.

It has also been stated that lupin seeds contain high concentrations of total tocopherols, which are commonly called vitamin E and have positive effects on human health (Hassanein et al., 2011; Briceno Berru et al., 2021). This tocopherol content in lupins significantly varies according to locations and genotypes (Annicchiarico et al., 2014).

The phenolic compounds in lupins, especially isoflavones, vary according to the sowing season (D'Agostina et al., 2008) and processing conditions (Khan et al., 2015). In this sense, especially germination leads to significant differences in phenolic compounds (Khan et al., 2015), and an increase in antioxidant activity is observed due to the increased concentrations of some secondary metabolites during the germination process (Dueñas et al., 2009; Rumiyati et al., 2013).

## CONCLUSION

In this article, the secondary metabolites of some legume forage crop species were examined, and their potential as functional foods and nutraceuticals for animal and human health was evaluated based on scientific evidence. *Medicago* sp., *Onobrychis* sp., *Melilotus* sp., and *Lupinus* sp. species with high nutritional value and forage quality are important sources of secondary metabolites in the plant kingdom. The secondary metabolites of these species have a wide variety of structures and chemical properties, and in addition to their important roles in the appreciation of the species as forage crop, they have important functions in plant physiology, especially in the defense mechanism against some diseases in ruminant animals, and in the yield and quality of animal products. The consumption of resources containing these phytochemicals may also play an important role in protecting human health. However, it should not be forgotten that it is necessary to conduct considerably more specific studies on this subject.

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## **CHAPTER 3**

### **UTILIZATION OF MEDICINAL AND AROMATIC PLANTS IN ANIMAL NUTRITION**

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## **INTRODUCTION**

Plants used by humans as food, clothing, shelter, tools and weapons have a long history. In the periods when modern medicine was not yet developed, people used plants effectively especially against diseases (Cook, 2011). Plants have been used in the treatment of diseases and the protection of public health since ancient times (Barata et al., 2016). It has been determined that 250 plants are used for therapeutic purposes by humans in the B.C. 5000s (Göktaş and Gidik, 2019).

However, increasing drug use due to advances in modern medicine has led to the emergence of different problems in humans. Especially the drugs used in more than the required doses can cause great harm to the human body. In recent years, people have been using medicinal and aromatic plants to protect themselves from these harmful effects of drugs.

Today, medicinal and aromatic plants have a wide range of uses, especially in food, cosmetics, paint, textile and medicine. Thanks to the increasing tendency of people to natural resources and nature, the demand for these plants is increasing day by day (Yılmaz and Karik, 2022). According to the World Health Organization (WHO) data, medicinal and aromatic plants appear as the main component in the traditional health system of many countries (Barata et al., 2016). The development level of countries is an important factor affecting the use of plants for therapeutic purposes. Resources show that 80% of people in developing countries are treated with herbal products, and this rate



rises to 95% in some countries in the Middle East, Asia and Africa. In developed countries, it is stated that the use of plants for therapeutic purposes is relatively low (Göktaş and Gidik, 2019).

Synthetic drugs and chemicals bring out problems for livestock as well as humans. While chemicals mixed with feeds cause problems in animals, they pass to humans through products used by humans such as meat, milk and eggs, and indirectly affect human health negatively. For this reason, the use of medicinal and aromatic plants as animal feed additives has increased in recent years.

In this review, informations about the use of medicinal and aromatic plants in animal nutrition is given.

## **1. GENERAL FEATURES OF MEDICINAL AND AROMATIC PLANTS**

It is not possible to define the medicinal plants exactly. Today, the terms "medicinal" and "aromatic" plants are often used together. Medicinal and aromatic plants are generally expressed as plants used as medicine to prevent and cure diseases and maintain health (Temel et al., 2018).

However, to explain in more detail; the term medicinal plant is used for plants that can be used for treatment in one or more organs or contain substances that are precursors for the synthesis of useful drugs. This explanation makes it possible to distinguish between medicinal plants whose therapeutic components have been scientifically determined, and plants that are considered to have medicinal value but

for which extensive scientific research has not yet been conducted (Sofowora et al., 2013). Aromatic plants, on the other hand, mostly cover products that are widely used in the production of spices and perfumes. In addition, aromatic plants provide raw materials for production of many important industrial chemicals (Devika, 2022).

While classifying medicinal and aromatic plants, they are grouped according to different criteria. The classification made by Pandey et al. (2020) are listed in Table 1.

**Table 1.** Classification of medicinal and aromatic plants

According to	Classification
Importance	Major aromatic plants, Minor aromatic plants
Use part	Herbage, root, wood, bark, leaf, flower, flowering tops, fruit, seed
Growth Habitat	Grasses, Shrubs, Herbs, Trees
Habitat	Tropical area, sub-tropical area, temperate area
Crop-duration	Annual, biennial, perennial
Method of propagation	Vegetative, Sexual (seed), both
Botanical	Division: embryophyta,
Classification	Sub division: gymnospermae and angiospermae

Medicinal and aromatic plants contain active substances such as essential oils, flavonoids, alkaloids, minerals and nutrients in different organs such as leaves, roots, flowers, fruits and seeds. Thanks to these substances they contain, they have therapeutic and nutritional properties (Çoban and Patır, 2010; Alamgir, 2017; Rahmati et al., 2022)

Medicinal and aromatic plants contain some substances with volatile properties. These substances, which are defined as essential oils, are obtained from parts of medicinal and aromatic plants such as flowers, buds, seeds, leaves, branches, fruits and roots. Essential oils

obtained by different methods (distillation, expression and fermentation) can contain more than 100 different compounds. The main components make up 85% of the essential oils, while the remaining components are only in trace amounts (Miguel 2010). The parts of some plants with medicinal and aromatic properties and the active substances contained in these parts are given in Table 2 (Adıyaman and Ayhan, 2010).

**Table 2.** Part use and active ingredients of some medicinal plants

Plant name	Part use	Active ingredient
Clove	Flower	Eugenol
Cinnamon	Shell	Cinnamaldehyde
Coriander	Leaf,seed	Linalol
Cumin	Seed	Cuminaldehyde
Anise	Seed	Anothole
Parsley	Leaf	Apiol
Black pepper	Fruit	Piperine
Ginger	Rhizoma	Zingorole
Garlic	Onion	Alicin
Rosemary	Leaf	Cineole
Oregano	Whole plant	Thmol, Carvacrol
Sage	Leaf	Cineole
Laurel	Leaf	Cineole
Mint	Leaf	Menthol

## 2. THE STATUS OF MEDICINAL AND AROMATIC PLANTS IN THE WORLD

Although the number of plants in the world is uncertain, it is estimated to be around 320.000-400.000 (Le Roux et al., 2017). It is reported that there are many plant species that have not yet been identified among these plants, and the number of identified ones is about 270.000. According to the World Health Organization (WHO)

data, around 20.000 plants are widely used for medicinal purposes around the world. While China has the highest number of medicinal and aromatic plants with 4941 plants, it is followed by India (3000), United States of America (2564), Vietnam (1800), Thailand (1800) and Pakistan (1500) (Keykubat, 2016). Considering the main trade centers of herbal medicines in the world, China again ranks first. A system that specifically classifies medicinal and aromatic plants does not exist yet. For this reason, there are uncertainties about world production and trade figures. When we look at the production of medicinal and aromatic plants in the world, it is seen that most of them are collected from nature (Pakdemirli et al., 2021).

Production areas and quantities of a significant part of medicinal and aromatic plants around the world are given in FAO statistics under the title of herbal production, mixed with other herbal products. Green coffee, cocoa, tea, flax and buckwheat rank first in terms of the size of their production areas (Temel et al., 2018).

It has been observed that data on the trade of medicinal and aromatic plants and products made from these plants are included in the Comtrade (International Trade Center-ITC) database as of 2001 (Boztaş et al., 2021). According to this database, while the export value of medicinal and aromatic plants and their products (1-Coffee, tea, maté and spices, 2-Medicinal plants, 3-Lac; gums, resins, saps and extracts, 4- Essential oils) was 65 billion dollars in 2011, it was increased to 69 billion dollars in 2020. The same situation has emerged in the import

value, and the export value, which was 64 billion dollars in 2011, increased to 67 billion dollars in 2020 (Türkecul and Yıldız, 2021).

### **3. THE STATUS OF MEDICINAL AND AROMATIC PLANTS IN TÜRKİYE**

Türkiye has a great biodiversity because of located between the temperate zone and the subtropical zone. Also, being located at the intersection of Asia, Europe and Africa, diversity of geographical and topographic features, elevation differences, Euro-Siberian, Mediterranean and Iran-Turanian phytogeographic regions and their transition zones, and having a wide variety of ecosystem types contribute greatly to the richness of Türkiye's biodiversity. In Türkiye, which is one of the richest countries in Europe and the Middle East in terms of biodiversity, the number of flora elements distributed is close to the number of plant species distributed throughout the European continent. Türkiye is home to around 12.000 plant taxa and 3000 of these plants are endemic. With its 34.4% endemism rate, it is one of the richest countries in Europe in terms of endemic species diversity (Seven, 2020). Türkiye is a very important country due to its biodiversity, and the basis of its medicinal and aromatic plant wealth is based on this. When the export statistics are examined, the fact that many medicinal and aromatic plants are included in the said statistics confirms this situation (Kırıcı et al., 2020).

The vast majority of medicinal and aromatic plants in Türkiye are generally obtained by collecting from nature. For this reason, reliable statistical data about these plants cannot be kept. In addition, the

medicinal and aromatic properties of many plant species in the spice and seasoning class make it difficult to set definite boundaries between the plants in question (Kurt and İmren, 2018). However, it is stated in different sources that 10.000 plant species grow naturally in our country and 500 of them are used for medicinal purposes. In addition, it is stated that about 200 species with medicinal and aromatic properties are exported (Gürbüz and Karahan, 2014; Samet and Cikili, 2015; Türkekul and Yıldız, 2021)

Around 300-350 medicinal and aromatic plants are sold in herbalists in our country (Faydaoğlu and Sürücüoğlu, 2011; Öztürk et al., 2012). When these plants are classified at the family level, the 7 largest families in terms of taxa numbers; Lamiaceae (18 taxa), Asteraceae (18 taxa), Apiaceae (11 taxa), Liliaceae (9 taxa), Rosaceae (8 taxa), Ranunculaceae (7 taxa) and Fabaceae (6 taxa). The genera with the most species are *Sideritis* (10), *Helichrysum* (8), *Rumex* (6), *Astragalus* (5), *Euphorbia* (5), *Gypsophila* (5), *Juniperus* (5), *Anthemis* (5), *Artemissia* (5), *Orchis* (4) and *Colchicum* (4). 73 of these plants are used externally and 168 are used internally for treatment (Öztürk et al., 2012).

The export value of medicinal and aromatic plants (1-Coffee, tea, maté and spices, 2-Medicinal plants, 3-Lac; gums, resins, saps and extracts, 4- Essential oils) in Turkey was 152 million dollars in 2011. In 2020, it increased to 350 million dollars. Likewise, the import value, which was 179 million dollars in 2011, reached approximately 450 million dollars in 2020. As in the world market, a significant increase

in both the export and import values of medicinal and aromatic plants draws attention in our country (Türkekul and Yıldız, 2021).

#### **4. USAGE AREAS OF MEDICAL AND AROMATIC PLANTS**

The lives of humans and plants are more intertwined than is often realized. Some plants have the power to alter the physiological functioning of our body. The use history of these plants, which are expressed as medicinal and aromatic plants, dates back to the beginning of humanity. People have been using these plants since ancient times. In addition to their great contributions in the field of medicine in terms of human health, they are also a source of financial gain for the producers who raise them. Medicinal and aromatic plants have a wide range of uses such as medicine, food, paint, perfumery and chemical industry (Aliu and Aliu, 2017).

Medicinal plants constitute a very important source of many components of vital importance to humans. Many plant species provide great benefits in the treatment of different diseases. Since ancient times, people have been using these herbs to prepare a home remedy for ailments such as seasonal flu, colds, cough, stomachache and headache. Their use has become widespread due to their high positive effects and low side effects, as well as being cheap and useful (Walia et al., 2022). The importance of these plants has emerged once again in the coronavirus pandemic we are in. Kadioğlu and Kadioğlu (2021) determined that the consumption of medicinal and aromatic plants increased by 76% due to the coronavirus epidemic, and plants such as

rosehip, linden, mint and ginger were widely used during the pandemic period.

Many herbs with medicinal and aromatic properties (anise, fennel, basil, mint, tarragon, marjoram, rosemary, thyme, parsley, juniper, laurel and black pepper) are widely used as spices in cooking. The aroma of these plants is obtained from essential oil components and essential oils have a strong antioxidant activity (Miguel, 2010).

In addition to the above-mentioned uses, medicinal and aromatic plants have also been widely used in animal nutrition in recent years. Plant parts and essential oils mixed into animal feeds have positive effects on the health of animals, especially due to their antioxidant effects, and provide better quality and healthier animal products. In this way, besides the direct positive effects of medicinal and aromatic plants on human health, indirect effects on animal foods also emerge.

## **5. USAGE OF MEDICAL AND AROMATIC PLANTS AS ANIMAL FEED**

Today, the human population is increasing rapidly. In parallel with the increase in population, adequate and balanced nutrition needs of people also increase. People obtain most of the nutrients they need in daily life from animal products. For this reason, the healthy production of animal products will protect the health of both animals and people who consume these products.

Livestock producers use many chemicals during production to protect animal health. The fact that antibiotics, hormones and hormone-



like substances used in the nutrition of ruminants cannot be metabolized in the body of animals immediately, they form resistance in bacteria and they pass into the human body with foods prepared from the meat of these animals after slaughter, causing serious problems for human health (Karayağız and Bülbül, 2014).

For this reason, natural preparations use recommended to instead of harmful chemicals to protect the health of both animals and humans. Medicinal and aromatic plants emerge as an important resource that can be used instead of chemicals in animal nutrition because of they have therapeutic properties and do not pose a risk to health.

Medicinal and aromatic plants have biological activities such as antioxidant, fungicidal and antimicrobial activities. Essential oils are the most concentrated form of phytobiotics that are increasingly used in the animal feed industry (Tajodini et al., 2014). Plant-derived products such as essential oils, herbs, and oleoresins are rich in phytobiotics. Phytobiotics, which improve feed properties and promote the production performance of animals, also increase the quality of products obtained from animals. For that reason, it is mixed with animal feeds, especially in commercial livestock (Mohsen and Kim, 2018). Phytobiotics have biological and aromatic properties. It can either be added directly to animal feeds or used in the form of extracts obtained from these plants (Karakci et al., 2022)

### **5.1. Poultry Nutrition**

Until recently, antibiotics were added to the feed of broiler chickens as feed additives in the world and in our country to encourage development. Also, antibiotics have been used for decades to reduce pathological infection in poultry feeding (Mohammed, 2018). However, the negative effects of the antibiotics caused their use to be banned. Poultry producers have used medicinal and aromatic plants as an alternative to antibiotics. Studies have shown that medicinal and aromatic plants have antibacterial, antiviral, antioxidant, antilipidemic, and antifungal effects. Positive effects of medicinal and aromatic plants on poultry; It can be listed as killing pathogenic microorganisms that may develop in the digestive organs, preventing the development of toxins in feed, increasing the activity of digestive enzymes and strengthening the immune system. As a result of these positive effects, the performance of broiler chickens improves (Adiyaman and Ayhan, 2010).

Plant extracts, in other words phytogetic feed additives, are used in poultry rations because they have properties that improve feed properties, increase animal performance and improve the quality of animal products (Gürsoy, 2021). Pathogenic bacteria such as *Escherichia coli*, *Salmonella* ssp, *Clostridium perfringens* and *Campylobacter sputorum*, to which animals, especially poultry, are highly sensitive, compete with the host for nutrients in the small intestine. These pathogens also reduce the digestion of fat and fat-

soluble vitamins by preventing the binding of bile acids to the substances they act on (Çimrin, 2018).

A study conducted by Abd El-Ghany and Ismail (2014) on chickens revealed that, according to the results of cell-mediated and humoral immune responses, essential oils with phytobiotic properties have an immune-enhancing effect when used together with ciprofloxacin. In the same study, it was reported that the effect of thyme essential oils on the stimulation of immune responses in chickens was somewhat low, but substances such as thymol and carvacrol had strong antioxidant properties that increased the immune responses of the chicks.

In another study, it was determined that thymol, which is high in the essential oil composition of thyme oil, passes into the egg yolk and exerts an antioxidant effect, and also increases egg production (Faydaoğlu and Sürücüoğlu, 2013).

Essential oils of medicinal and aromatic plants have performance-enhancing and antimicrobial effects for animals. Jamroz et al. (2005) reported that bodyweight was not enhanced in broilers with the addition of herbal extracts consisting of thyme (carvacrol), cinnamon (cinnamaldehyde) and black pepper (capsaicin) in diets based on maize or wheat and barley. In addition, the rate of feed conversion increased between 2% and 4.2%, reduction of *E. coli*, *Clostridium perfringens* and fungi and increase of *Lactobacillus* spp. were observed. It is reported that the number of lipases increases and the lipase activity in the pancreas and intestinal wall increases.

Fawaz et al. (2022) reported that turmeric powder can be used as an effective feed additive to improve egg production, egg quality, nutrient digestibility, fecal ammonia concentration and blood biochemistry in laying hens between 55 and 67 weeks.

Garlic, which is one of the plants that is widely used as a spice and herb, is used by people for various scientifically approved reasons (inhibiting bacterial growth, reducing oxidative stress, etc.). In broiler farming, garlic can be added to feed as a natural feed additive, as it improves growth and feed efficiency and reduces mortality (Puvača et al., 2015).

In a study conducted to evaluate the effects of adding jojoba bioactive lipid compounds to broiler rations on growth performance and meat quality, it was determined that the growth performance of broiler chickens fed with jojoba seed oil in tropical hot climate conditions was better than the control group. In addition, it was determined that abdominal fat decreased, carcass percentage and meat quality increased in animals with jojoba seed oil supplementation (Abdel-Wareth et al., 2022).

Daş et al (2020) in a study they conducted to investigate the effects of peppermint oil added to quail rations on growth performance, meat quality, color and blood oxidative stress characteristics; They reported that peppermint oil added to the rations did not affect the fattening performance and carcass characteristics, but increased the total antioxidant values and decreased the total oxidative values,

therefore it would be beneficial to add 0.1% peppermint oil to the ration as a feed additive.

Ramteke et al. (2022) reported that coriander seed powder added to chicken rations is a good source of crude protein for both extract and total ash and can be used as a phyto-genic feed additive because it does not cause any adverse effects.

## **5.2. Ruminant Nutrition**

Ruminants (especially livestock) have a very important place in human nutrition. 70% of total animal protein and 10% of natural fiber consumed by humans are provided by these animals. Ruminants have symbiotic populations of rumen microbes and the ability to chew partially digested food. For this reason, they have the capacity to use roughage with high fiber content that cannot be consumed by humans (Minson, 1990).

Feed quality and quantity are very important in order for rumen activities to be carried out at optimum level in ruminants. Since this is not always possible, feed additives that protect animal health by improving rumen conditions and digestibility of feeds have been started to be used for the continuity of yield (Gürsoy, 2021). However, since some of the feed additives are chemical affects both animal health and the quality of the products obtained from these animals, manufacturers have turned to more natural feed additives. Medicinal and aromatic plants constitute a very important alternative in this regard. Different parts and active ingredients of many medicinal and aromatic plants are used as feed additives and provide great benefits in terms of animal

nutrition. Some of these plants are given in Table 3 with their used parts, active component and functions (Frankič et al., 2009).

Oxygenated monoterpenes, especially monoterpene alcohols and aldehydes, can strongly inhibit the growth and metabolism of rumen microbes. In addition, monoterpene hydrocarbons slightly inhibit and sometimes stimulate the activity of rumen microbes. This information shows that the chemical composition of essential oils greatly influences their effects on the activity of rumen microorganisms (Benchaar, 2009).

While essential oils obtained from medicinal and aromatic plants affect the cell membranes of gram-negative bacteria, they can act within the cells of gram-positive bacteria. It is thought that essential oils that are active against gram-negative bacteria contain very small secondary metabolites, so they can pass through the porin proteins in the outer membrane of the bacteria and reach the plasma membrane (Kmicikewycz, 2011). Thymol breaks down the outer shell of gram-negative bacteria, causing the release of lipopolysaccharides and increasing the permeability of the cytoplasmic membrane for ATP (Kestane, 2019). Bampidis et al. (2006) reported that thyme oil has the ability to completely inhibit the growth of *E.coli* in vitro, and the dried thyme leaves applied as an oral solution to calves with diarrhea (Neomycin: An aminoglycoside antibiotic active against both gram-positive and gram-negative bacteria (Allport et al., 2022) stated that it can affect fecal scores.

**Table 3.** Used parts, active component and functions of some medicinal and aromatic plants used as feed additives

Plant	Used parts	Active component	Functions
Cinnamon	Bark	Cimetaldehyde	Appetite and digestion stimulant, antiseptic
Cloves	Cloves	Eugenol	Appetite and digestion stimulant, antiseptic
Cardmom	Seed	Cineol	Appetite and digestion stimulant
Coriander	Leaves, seed	Linalol	Digestion stimulant
Cumin	Seed	Cuminaldehyde	Digestive, carminative, galactogogue
Anise	Fruit	Anethol	Digestion stimulant, galactogogue
Celery	Fruit, leaves	Phtalides	Appetite and digestion stimulant
Fenugreek	Seed	Trigonelline	Appetite stimulant
Capsicum	Fruit	Capsaicin	Digestion stimulant
Pepper	Fruit	Piperine	Digestion stimulant
Mustard	Seed	Allyl izotiocianat	Digestion stimulant
Ginger	Rizom	Zingerone	Gastric stimulant
Garlic	Bulb	Alkin	Digestion stimulant, antiseptic
Rosemary	Leaves	Cineol	Digestion stimulant, Antiseptic
Thyme	Whole plant	Thymol	Digestion, stimulant, antiseptic, antioxidant
Mint	Leaves	Menthol	Appetite and digestion stimulant, antiseptic

Anthelmintic (worm-reducing) drugs used to control parasites in the digestive systems of animals pose a risk to people using products derived from these animals. For this reason, the use of medicinal and aromatic plants with anthelmintic effects provides great benefits in this regard. For example, while the *Lanata camara* plant has an important place in the control of parasites and nematodes in the digestive system, Eucalyptus species are known to have an anthelmintic effect in goats (Faydaoğlu and Sürücüoğlu, 2013).

Essential oils obtained from medicinal and aromatic plants have the potential to be natural growth promoters in calf nutrition. Therefore, it is thought that adding essential oils and their mixtures to calf diets will benefit calf health and performance (Bal and Selçuk, 2021). Selvi (2018) examined the use of thyme oil in calves drinking formula and determined that the addition of thyme oil to the food had a positive effect on the slaughter age, total live weight gain and daily live weight gain. It was concluded that the calves that were given thyme oil were separated from the food at an early age, and this was due to the fact that thyme oil increases feed consumption and appetite, accelerates rumen development, regulates rumen pH and is effective against harmful microorganisms in the rumen.

A study on lambs showed that oregano essential oils mixed into feeds increased the amount of total volatile fatty acids in the rumen fluid and had the potential to improve rumen fermentation. This situation revealed that essential oils can be used as a rumen fermentation modifier in lambs (Baruh and Kocabağlı, 2017).

Şengezer and Güngör (2008) reported that the use of essential oil in dairy cows significantly prevented the deamination rate in the rumen, while the use of cinnamon, garlic, thyme and anise as feed additives increased the acetate and butyrate ratios in the rumen, while decreasing the propionate ratio. The same researchers noted that the effects of plant extracts on ruminal fermentation in beef cattle may vary depending on ruminal pH and provide more energy efficiency, and they stated that



these extracts can also be used to prevent environmental pollution caused by farm animal manures and pathogenic microorganisms.

Önenç et al. (2019), in a study examining the effects of thyme and cumin essential oil addition on fermentation quality, aerobic stability and in vitro metabolic energy contents of alfalfa silage, it was determined that essential oils added at the level of 650 mg kg<sup>-1</sup> promote silage fermentation and improve aerobic stability. In addition, thyme essential oil was reported to be more effective in preventing mold growth and cumin essential oil was more effective in preventing yeast growth. On the other hand, Kepekçi and Duru (2020), determined that anise seed mixed into alfalfa silages caused an increase in fermentation quality, especially with the addition of 0.5% anise seed, the pH value decreased and the ammonia nitrogen content decreased numerically. According to another data obtained in the study, it was concluded that the addition of anise can have a positive effect on the long-term preservation of silages.

### **5.3. Effects of Medicinal and Aromatic Plants on Methane Gas Production in Animals**

Methane is a greenhouse gas that causes global warming and its effect is 23 times stronger than carbon dioxide gas. This gas, which is also produced by ruminant animals, is responsible for the loss of 2-12% of the gross energy obtained with the feed. Therefore, reducing methane produced by animals not only increases nutrient use efficiency, but also helps protect the environment from global warming (Bhatta et al., 2013). Delgadillo-Ruiz et al. (2021) stated that the use of essential oils

in animal nutrition should be investigated, given the concerns about the production of greenhouse gases that cause global warming, especially methane emitted from ruminant animals. Because essential oils, thanks to their antibacterial effect, affect the rumen microbiota, change the fermentation and reduce the methane concentration.

Arhab et al. (2013) were performed a study to determine the *Satureja calamintha*, *Mentha pulegium* and *Juniperus phoenicea* on in vitro methanogenesis and fermentation traits of vetch-oat hay. The results of the study showed that methane (CH<sub>4</sub>) production and ammonia nitrogen concentrations decreased at all doses of the three essential oils tested. Researchers reported that secondary metabolites have the potential to reduce methanogenesis in the rumen, but more in vitro and in vivo trials are required to determine the optimum dose that reduces methane (CH<sub>4</sub>) production without adversely affecting fermentation and rumen functions.

Bhatta et al. (2013) in a study they conducted with three plants (*Canthium inerme*, *Gymnema sylvestre* and *Sapindus laurifolia*) that have medicinal and aromatic value and contain tannins, they stated that tannins prevent fiber degradation, reduce the population of protozoa and methanogenic archaea, and thus suppress in vitro methanogenesis. Garcia-Gonzales et al. (2008) determined that the rhizomes and roots of *Rheum officinale*, the bark of *Frangula alnus* and the bulbs of *Allium sativum* reduced both methane production and acetate propionate ratio. Sallam et al. (2011) reported that essential oils obtained from the leaves of *Mentha microphylla* and added to the rumen fluid provide a

significant reduction in the actual degradation of dry matter and organic matter, while reducing the number of protozoa and  $\text{NH}_3\text{-N}$  concentration. When the same researchers evaluated the obtained results, they concluded that the essential oil obtained from *M. microphylla* can be used as a methane reducing agent.

#### **5.4. Feed Value of Medicinal and Aromatic Plants**

Meadows and pastures are the most important source of roughage for animal feeding. In these areas, there are plants with medicinal and aromatic properties as well as forage plants. These plants are grazed by animals, especially by small cattle, so that both they are fed and gain resistance against diseases (Balabanlı et al., 2019).

Başbağ et al (2018), as a result of a study they conducted in 13 different plant species (*Mentha spicata*, *Origanum onites*, *Thymus kotschyanus*, *Salvia officinalis*, *Artemisia* sp., *Aloysia citriodora*, *Melissa officinalis*, *Tanacetum densum*, *Ocimum basilicum*, *Satureja hortensis*, *Mentha piperita*, *Thymbra spicata* and *Phlomis kotschyana*) reported that the plants have the potential to produce high quality forage. Researchers also emphasize that the grasses of these plant species contain both toxic and mineral substances, therefore, it is necessary to pay attention to the negative effects that may arise from the intensive consumption of animals. Başbağ et al. (2020), on the other hand, reported that the crude protein ratio and digestibility were high, while the macro-mineral content and  $\text{K}/(\text{Ca}+\text{Mg})$  ratio were at sufficient levels in their investigations of the dried herb of the *Salvia multicaulis* species collected from different locations.

Lupu et al. (2019) in a study in which they investigated the chemical compositions of 3 medicinal and aromatic plants (Mint (*Mentha longifolia*), fennel (*Foeniculum vulgare*) and white willow bark (*Salix alba*)) found that mint and fennel were found in significant proportions (20.72% and 18.89%, respectively). They determined that it contains crude protein and that fennel seeds are rich in crude fiber (27.31%). In addition to these, it has been determined that the plants are also rich in mineral substances. When the researchers evaluated the results obtained from the study, they stated that the mechanism of action of the phytoadditives could not be fully understood, but based on the chemical composition of the plants studied, some of them could be used in animal nutrition, especially mint, which is a very important source of trace minerals, essential amino acids and  $\Omega 3$  fatty acids.

### **5.5. Negative Effects of Medicinal and Aromatic Plants on Animals**

Rumen bacteria in ruminants have intracellular and extracellular destructive and constructive enzyme systems. Animals hosted by bacteria cannot synthesize these enzymes or these enzymes are not found in the rumen. Bacteria attack the nutrients in the rumen in order to survive with the help of these enzyme systems and form their own body tissues by meeting their energy needs (Özel and Sarıçiçek, 2009). Tannins have a toxic effect on rumen bacteria and cause morphological changes in ruminant animals. They also reduce the protein and carbohydrate intake of animals. Essential oils, which are expressed as substances with a special smell and can be entrained with water vapor,

are usually liquid and almost colorless when fresh. Animals do not eat plants containing these oils because of their strong odor and burning properties. In case such plants are eaten by animals, vomiting and bleeding may occur as a result of irritation in the digestive tract mucosa (Kitiş, 2012).

Especially in meadows and pastures, there are plants that grow naturally and are risky for animals due to the toxic substances they contain. These plants prevent the grass produced in meadows and pastures from being used well by animals, and negatively affect the quality and quantity of animal products by causing loss of appetite. There are even plants that sometimes cause the death of animals when consumed in large quantities. The toxic effects of these plants, which are expressed as poisonous plants, on animals can vary according to the seasons and even months. E.g; *Delphinium* spp. at the end of spring and beginning of summer, *Conium maculatum* in sunny summer months, *Hypericum perforatum* causes poisoning of animals in every period during vegetation. Age and breed of animals also play an important role in poisoning events. Generally, old animals do not eat poisonous plants easily because they recognize them, but young animals do not have the same sensitivity. In addition, native breeds are more resistant to poisoning than cultural breeds (Balabanlı et al., 2006).

Kara and Sürmen (2019) recommend attention to the following situations in order to protect animals from poisoning;

- Recognition of poisonous plants growing in pasture.
- Under what conditions plants can be dangerous for animals.

- Developing a grazing plan to improve pastures and prevent animals from being poisoned by plants. Grazing of pastures on time and in accordance with grazing capacity.
- Not grazing animals that are under stress or starving in areas where poisonous plants are concentrated.
- Providing adequate water for animals.
- Paying attention to the animals that will graze for the first time in the pasture.
- Providing adequate salt and other supplements as needed.
- Control of poisonous plants where possible.

If animals become ill due to any plant, the veterinarian should be consulted without delay. It is very important to identify the plant correctly for rapid diagnosis and treatment during the application.

## **6. CONCLUSION**

Today, medicinal and aromatic plants, which are widely used in food, cosmetics, paint, textile and medicine, have a great importance in terms of human health thanks to the secondary metabolites they contain. Since ancient times, people have used medicinal plants to cure diseases. In recent years, the demand for medicinal and aromatic plants has increased considerably due to the side effects of the chemical drugs used. The active ingredients of these plants provide great benefits not only for humans but also for the animals whose products we use. Especially the prohibition of antibiotics used in animal nutrition due to their side effects has directed manufacturers to different feed additives, and medicinal and aromatic plants have created an important alternative

in this regard. Medicinal plants used in poultry farming, especially due to their antioxidant properties, positively affect both meat and egg quality. Likewise, medicinal and aromatic plant preparations mixed with the feed of ruminants have positive effects on the rumen flora of animals, thus increasing the performance of animals and obtaining higher quality animal products. These plants also contribute to the protection of the environment by reducing the production of methane gas in animals. In addition to these positive features of medicinal and aromatic plants, there are also some negative aspects. For example, poisoning cases can be encountered when animals grazing on pastures eat plants that grow in the natural flora of the rangeland, especially contain alkaloids. For this reason, poisonous plants in the pastures should be followed and animals should be prevented from grazing these plants.

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## **CHAPTER 4**

### **HERBAL TREATMENT METHODS USED IN CHRONIC DISEASES**

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## **INTRODUCTION**

Chronic diseases generally refer to diseases that last a year or more. It is defined as situations that require continuous medical attention and limit daily life activities or require both (WHO, 2014). The Commission on Chronic Illness (CCI; Commission on Chronic Illness) is defined as "mostly non-infectious diseases, often involving socio-economic, personal and genetic factors, which are not fully recovered, are persistent, often permanent disability" (Roberts, 1954). The reasons for chronic diseases today are: Development of diagnostic and treatment methods over time, increased importance to preventive health care, increased life expectancy and growing aging of the world's population, the struggle against infectious diseases in many countries, inadequate sleep, physical inactivity, psychological stress, environmental pollution, there are many factors, such as cigarette or alcohol abuse, unhealthy lifestyle that arising from unhealthy nutrition (Steyn and Albertino, 2006). Chronic diseases are often slow-developing, sneaky-looking, and do not show sudden changes during their course, long latent period. In the formation of these diseases, genetic, environmental and socio-economic factors, cultural and individual characteristics are very important. On the other hand, as the older population increased in proportion to the younger population, health problems in society have led to their childhood diseases, which have caused them to shift from infectious chronic diseases in the elderly population (Sassi and Jeremy, 2008). In the process of the formation of chronic diseases, the disease can become more severe over time, and it can also create a different clinical picture that becomes more violent



than slightly. Although the disease sometimes becomes a healing phase, repetitive paintings can make the disease worse. However, an existing chronic disease can also cause a different chronic disease. These individuals need hospital and community care based on the period and severity of their illness. Patients are treated at the hospital during acute or flare-up periods, while their primary treatment and care is carried out where they live. How these people are treated, how they perceive the disease, how they put it into the disease, and how they react to it, affect the disease adaptation process and treatment. The adaptation of the individual to the disease is influenced by factors such as age, psychosocial status, denial of disease, confidence in himself (Karakoç and Taşkın, 2014).

Chronic diseases are the leading cause of death and disease worldwide in all World Health Organization (WHO) regions except Africa (WHO, 2005). Early deaths caused by chronic diseases are among the serious problems for public health. Approximately 45% of chronic disease deaths and 86% of the chronic disease burden occur in people under the age of 70 and 15 million people die every year between the ages of 30-70 and (WHO, 2018). Today, chronic diseases such as cardiovascular diseases, respiratory diseases, diabetes and cancer are common in society and affect all communities (WHO, 2010). Cardiovascular diseases continue to be a major cause of death in many industrialized countries and are growing alarmingly in developing countries (Yusuf et al., 2001). It comes first in the cause of death due to chronic diseases and is responsible for the deaths of 17.9 million people

in 2018. Cardiovascular diseases account for 44% of the deaths caused by chronic diseases and 31% of all global deaths. Cancer is the second worldwide cause of death (8.97 million deaths) after cardiovascular disease. However, it is estimated that in 2060, deaths from cancer (~18.63 million deaths) will be the first. Cancer is 9% of the deaths caused by chronic diseases and 16% of all global deaths (WHO, 2016). In third place, respiratory diseases caused the deaths of 3.8 million people by creating 9% of all chronic diseases and 7% of all global deaths. Diabetes, on the other hand, is the death of 1.6 million people, as well as 4% of the deaths caused by chronic diseases, and 3% of global deaths (WHO, 2019).

According to Türkiye's Statistical Institution (TÜİK) data in 2019, the causes of death among the elderly were reported to be first-place circulatory system diseases (41.5%), second-place benign and malignant tumors and respiratory system diseases (15.3%), third, neurones and sensory organs (5.3%) (Anonymous, 2021). WHO has stated that 4.5 million people will die by 2030 due to Chronic Obstructive Lung disease worldwide (WHO, 2021). In the 21 st century, with advances in medicine and technology, the diagnosis and treatment methods of chronic diseases have improved, the importance of preventive health services has increased, and there has been a significant reduction in mortality rates due to diseases. However, the number of people with chronic diseases is increasing (Yeşil et al., 2016). Chronic diseases are not only the problem of developed countries, but the leading cause of death for all countries. More than

80% of deaths in low and medium-income countries are seen due to chronic diseases (WHO, 2021). In addition to the deaths caused by chronic diseases, the country is putting serious burden on its economies. The average 60-80% of total health expenditures are divided into chronic diseases. While diagnosis and treatment of these diseases is very costly, diagnosis and treatment of cancer, diabetes, hypertension patients in particular increases the cost (Kara, 2011). Economic load analysis shows that health care expenditures related to chronic diseases are only the visible face of the iceberg. In 2016, the total cost of chronic diseases to the Turkish economy was set at 69.7 billion TL.

As a result, the number of individuals with chronic disease is increasing and is applying various methods for treatment of the disease. One of the ways that individuals with chronic diseases have been treated is a herbal treatment method. In Turkey, more and more interest in the use of herbal treatment for treatment of chronic diseases is increasing. The religious beliefs of individuals, socio-cultural structures, lifestyles and beliefs that they will find healing affect their orientation to herbal treatment. In addition, the idea that individuals will have less impact on their health by using herbal treatment methods will be cheaper and less likely to have a side effect increases their use of herbal products.

## **1. CANCER AND HERBAL TREATMENT METHODS**

Cancer is the process of turning these cells into malignant, tumor cells through many stages after a small change in normal cells. The word meaning of cancer is that it is malignant urges that occur in an organ or by the uneven split of cells in tissue. Cancer cells are known

to multiply and form over 100 groups of diseases. Although the cancers are very varied, they all begin with abnormal reproduction in cells in general. If they're not treated, they could cause serious problems and eventually cause death. The frequency of cancer can also vary depending on the geographical area and race. Also, cancer types can be seen in men and women in different frequency. The most common type of cancer in men and women is skin cancer. In Turkey, the most common number of cancer types in men are lung, prostate, thick bowel, rectum, stomach and pancreatic cancer. The most common types of cancer in women are breast, lungs, thick bowel, rectum, cervical, over, stomach and pancreatic cancer (Ministry of Health of the Republic of Türkiye, 2022).

In cancer treatment, early diagnosis and screening programs, adaptation to treatment and dealing with disease strategies, culture, belief and the impact of values are known (Navon, 1999; Schulmeister, 1999). Factors such as physical activity, nutrition, clothing, etc. have a significant impact on the formation and development of cancer cells. Materials used to prepare foods (smoked, salamura.), overconsumed foods (oily, carbohydrate, etc.), alcohol and cigarette use, fatality, sedanter life, etc., affect health in a negative way (Itano, 2011).

Patients diagnosed with cancer experience feelings such as uncertainty and anxiety about the future, on the one hand, and fears due to the side effects of chemotherapy/radiotherapy on the other hand. Many chemotherapeutic drugs or molecular targeted drugs are used in cancer treatment. The drugs used for cancer do not yet respond to the

desired level of treatment. After applying chemotherapeutic drugs, there are many side effects which cause patients to worry (Albeloff et al., 2004). It also reduces the quality of life in some patients, causing them to experience difficulty in performing their daily activities and problems in adapting to treatment. Some patients are unable to cope with these difficulties, but are considering taking a break from treatment or interrupting the treatment. This reduces the effectiveness of the treatments and causes the disease to advance further. In addition, the side effects of the treatments they receive, even if the patients complete their treatment, can continue for a long time, and therefore the patients are experiencing a period of distress (Çapar, 2010). For these reasons, patients use herbal treatment to deal with these symptoms and side effects to improve life and quality (Weiger et al., 2002).

**Carob (*Ceratonia siliqua*):** Carob, also known as the carob tree, includes many morphological changes as well as being a plant with a root system based on arid regions. Carob, which contains more than one flavonoid and carotenoid, is widely used for antioxidant purposes. Carob is good for bone-related problems due to its high potassium and calcium content. It also shows anti-oxidant, anti-bacterial, anti-diarrheal, anti-allergic and sedative effects (Mokhtari et al., 2012; Rasheed et al., 2019). Carob, which also contains vitamins E, D, C, Niacin, B9, Cobalamin and a high percentage of fatty acids, has antiproliferative and anticancer effects (Papaefstathiou et al., 2018). People living in the Western Black Sea region, with the thought that it is good for cancer and eczema, boil the carob leaves in water, strain

them, consume them as tea on their empty stomachs, or drink them as tea by mixing them with nettle leaves (Yeşilada, 1999). In the Eastern Black Sea region, carob fruit is consumed by boiling in water and drinking (Toksoy et al., 2010). It is reported in the literature that it has lung cleansing properties and even protective properties against lung cancer (Tuzlacı, 2016).

**Garlic (*Allium sativum*):** Garlic is among the plants in the oldest cultures. The average height of garlic is 25 to 100 cm. It is a culture plant with greenish white or pink flowers, herbaceous root and stem, and leaves, teeth and flower parts. Garlic is considered to be a strong anti-cancer agent due to its content, as it helps to strengthen and regulate the immune system; It destroys carcinogens that can cause tumor formation (El-Mofty, 1994). Studies have shown that there are large differences in cancer rates in regions that consume more or less garlic. It has been found that the risk of developing gastric cancer is less than half in regions where garlic is consumed more than those living in regions where it is consumed little or no garlic (Fleischauer et al., 2000). In the Central Anatolia Region, people drink a glass of warm water, mixed with a tablespoon of crushed garlic and lemon juice after they eat in the evening to treat cancer (Tuzlacı, 2006).

**Stinging Nettle (*Urticaceae*):** Nettle weed, the plant that grows in temperate regions, is widely grown in Asia, Europe and America (Vogl and Hartl, 2003). The whole plant is covered by burning hairs. These feathers can show spherical, masked, stellar, worm-like shapes and are used to describe them in some types. The incinerator and

scratcher feature of the bite is removed by heating or drying the grass. So the leaves of the cooking nettle can be easily consumed and have a nutritious value (Kurban et al., 2011). In the content of the nettle, potassium salt, histamine, acetyl-colin, vitamin C and formic acid have been proven to stop the growth of cells that cause cancer (Yavuz et al., 2007). In the Dereli district of Giresun, the leaves of netting grass are consumed by drinking three cups a day in the form of tea to treat cancer (Tuzlacı, 2006). People living in Kesan district of Edirne consume 1 to 2 cups of water each day by boiling the leaves of netting grass in water to protect against cancer (Yeşilyurt et al., 2017). In the Eastern Anatolian Region, the remaining parts of the plant are freshly eaten to protect or cure all kinds of cancer (Özgökçe and Özçelik, 2004).

**Turmeric (Curcumin):** Turmeric is obtained from a plant called “*Curcuma longa*”. Curcumin in the plant is used in the treatment of many chronic diseases caused by inflammation with its polyphenolic feature. Due to its antioxidant properties, turmeric is widely used among the public for the protection of health. It is stated that it prevents many types of cancer and helps in the treatment of cancer (Akbay and Pekcan, 2016).

## **2. DIYABETES MELLITUS AND HERBAL TREATMENT METHODS**

The WHO accepts diabetes mellitus, which is a very important place in chronic diseases, among the most important public health problems of the new millennium (WHO, 2013). In 2013, the International diabetes Federation estimates that 382 million adults

worldwide aged 20-70 have diabetes and 80% of diabetic influences live in low and middle income countries. This number is expected to increase to 592 million by 2035 (Abdul-Ghani et al., 2006).

Diabetes mellitus is defined as a group of metabolic diseases characterized by hyperglycemia caused by defects in insulin release or insulin effect or both. The chronic hyperglycemia effect of diabetes is associated with long-term damage, dysfunction and failure in various organs, especially the eyes, kidneys, nerves, heart and blood vessels (Diabetes Mellitus Report, 1997). Various pathological processes are involved in the development of diabetes. These extend from the autoimmune destruction of the pancreas  $\beta$ -cells to the abnormalities resulting in insulin deficiency and resistance to insulin effect. The basis of abnormalities in the metabolism of carbohydrates, fat and protein in diabetes is due to insufficient effect on the target tissues of insulin. The missing insulin effect is due to insufficient insulin secretion and/or decreased tissue response to insulin at one or more points in the complex paths of the hormone effect. Insulin secretion disorder and insulin-induced defects are often found in the same patient, and it is often unclear which abnormality alone is the primary cause of hyperglycemia (Diabetes Care, 1997; Diabetes Care, 2003). Significant symptoms of hyperglycemia include polyuria, polydipsi, sometimes polyphage, weight loss and blurred vision. Growth disorder and sensitivity to some infections can also accompany chronic hyperglycemia. Acute, life-threatening results of uncontrolled diabetes are ketoacidic hyperglycemia or nonketotic hyperosmolar syndrome



(Carpenter and Coustan, 1982). Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy that leads to kidney failure; periferik neuropathy with risk of foot ulcers, amputation and Charcot joints; and autonomous neuropathy that causes gastrointestinal, genitourinary and cardiovascular symptoms and sexual dysfunction disorder. The insidation of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease has increased in diabetic patients. Abnormalities of hypertension and lipoprotein metabolism are usually found in diabetic people (Carpenter and Coustan, 1982). The pathogenesis of most diabetes cases is divided into two categories. In a category, type 1 diabetes is the result of an absolute lack of insulin secretion. Individuals with a high risk of developing this type of diabetes can be identified by serological evidence and genetic markers of the autoimmune pathological process, usually occurring on pancreatic islands. The other category is type 2, which is a much more common category, and the reason is a combination of insulin resistance and inadequate compensating insulin secreting response. Prior to the second category of diabetes, there may be a long process that causes pathological and functional changes in various target tissues, but does not have clinical symptoms, and to some extent does not diagnose hyperglycemia and diabetes (Cho et al., 2002).

Various antidiabetic drugs have been applied in medical applications, but however, insufficient prevention of secondary complications and the occurrence of side effects have led to traditional therapeutic approaches (Vetrichelvan et al., 2002). Medicinal plants are

considered to be a giant source of biological active substances that play a key role in past and modern therapeutic approaches. Ethnobotanical evidence of herbal medications with potential antidiabetic activity has been reported for about 800 plants (Mentreddy, 2007).

**Mountain thyme (*Origanum oyriacum*):** Popularly, mountain thyme is widely used to lower blood sugar (Bulut, 2008). After the mountain thyme is turned into essential oil in the Balikesir and Mugla region, it is used to lower blood sugar by dripping a few drops into a glass of water. In the Koçarlı, Bodrum and Ezine regions, the leaves and flowering parts of the mountain thyme are used by mixing them to reduce blood sugar, as well as by preparing them from the above-ground part. It is also known that essential oil, called thyme oil, is used among the public to reduce blood sugar in diabetics by drinking it (Tuzlacı, 2016).

**Blackberry (*Rubus fruticocus*):** It is a bush called blackberry fruit or, more commonly, blackberries, and famous for its fruit. The fruit has medicinal, cosmetic and nutritious value. It is a concentrated source of valuable nutrients as well as bioactive components of therapeutic interest, which emphasizes its importance as a functional food. In addition to its use as fresh fruit, it is also used as a ingredient in cooked dishes, salads and bakery products such as jams, snacks, sweets and fruit cans. In blackberry oil; There are various pharmacological effects such as vitamins, steroids and lipids and antioxidants, anti-carcinogenic, anti-inflammatory, antimicrobial, anti-diabetic, anti-diarrhea. Blackberry plant grains; gallic acid, villosin and iron, vitamin

C, niacin (nicotinic acid), pectin, sugars and anthocyanins, albumin, citric acid, malic acid and pectin (Verma and Rubus, 2014). Blackberries are widely used to treat diabetes (Bailey and Day, 1989). In the Central and Western Black Sea regions, where the hypoglycemic effect of blackberries is known, it is known that the mixture in which the roots and branches of blackberries are boiled in water is filtered and consumed in the form of tea (Petrofsky et al., 2008). In Merzifon district of Amasya, diabetics consume the fruits of blackberries by boiling them in water (Ezer and Mumcu, 2006). In ethnobotanical studies carried out in Solhan district of Bingöl, it is known that in diabetes, tea prepared from the leaves of the plant is consumed one cup a day after eating (Polat et al., 2013).

**Purslane (*Portulaca oleracea*):** From the purslane family; It is an annual herbaceous plant. Its body is flat on the ground, its leaves are sessile and fleshy. The edible parts are small, round green leaves and delicate stems. It is used colloquially as purslane, parpar, pırpım, purpürüm, cibile, applelik, purslane, coldness, tohmegen (Koç, 2002). It is known that purslane is rich in vitamins, minerals, unsaturated fatty acids, especially omega-3 fatty acids, glutamic acid, glutathione and aspartic acid (Simopoulos et al., 1992). Polysaccharide (POL-P) extracted from purslane has hypoglycemic and hypolipidemic activities as well as antioxidant and antitumor activities (Zhao et al., 2013). Consumption of purslane raw or mixtures prepared with leaves with 1 glass of water once a day before meals are widely used among the people to lower blood sugar (Bulut et al., 2016).

**Sloe (*Prunus spinosa*):** It is a wild plum species that grows naturally in Europe, West Asia and Northwest Africa (Baytop and Byfield, 1977). The fruits of the sloe are bluish purple in color, the skin is greenish, fleshy and has large seeds. It has a sour taste. It contains organic acids, pectin, sugar, and its flowers; It is rich in flavones and glycosides. Fruits also have high potassium, calcium, magnesium, phosphorus, sulfur, sodium, iron and crude fiber content and contain a certain amount of selenium and zinc (Çalışır et al., 2005). Sloe is widely used in Datça to lower blood sugar by boiling the leaves in water after drying (Tuzlacı, 2006). In Çanakkale and Ezine, tea prepared from fresh leaves is consumed in the morning on an empty stomach, to reduce blood sugar as 1 tea glass. In the Antalya region, the use of fruits after boiling in water and straining in diabetes mellitus is recorded in the literature (Tuzlacı, 2006).

**Thorn Grape (*Berberis vulgaris*):** Thorn grape fruit contains dextrose, fructose, malic acid, tartaric acid, citric acid, pectin and resin. It is also rich in vitamins C and A, calcium, iron and potassium (Ezaei et al., 2011; Yin et al., 2002). It has been reported that thorn berry fruit extract is good in streptomycin-induced diabetes (Ashraf et al., 2013). In Adıyaman and other Eastern Anatolian provinces, the fresh fruits of the plant are consumed directly by diabetics (Öztürk, 2011; Furkan, 2016). In Erzincan, tea prepared with the roots of the plant is used by diabetics to lower blood sugar (Korkmaz and Alparslan, 2014).

**Jerusalem artichoke (*Helianthus tuberosus*):** Jerusalem artichoke is a tuberous plant rich in carbohydrates, 70-90% of which is

inulin (Bekers et al., 2007 ) and its tuber is edible. This plant provides much lower energy than sugar or grain. Since inulin cannot be digested by the human enzyme system, it is suitable for the nutrition of diabetics and patients who need special diets use it instead of sugar and flour (Barta and Rosta, 1958). Traditionally, the tubers of Jerusalem artichoke are consumed raw in the treatment of diabetes (Furkan, 2016; Bulut et al., 2016). In Kars, Bingöl and many Eastern Anatolian provinces, the stem part of the Jerusalem artichoke is consumed fresh by diabetes patients (Altundağ and Öztürk, 2011). In the Babaeski district of Kırklareli, the tuber parts are eaten and consumed in order to lower blood sugar (Tuzlacı et al., 2007).

**Quince (*Cydonia oblonga*):** Quince is widely rich in beneficial secondary metabolites such as phenolics, steroids, flavonoids, terpenoids, tannins, sugars, organic acids and glucosides. It has a wide range of pharmacological activities such as antioxidant, antibacterial, antifungal, anti-inflammatory, hepatoprotective, cardiovascular, antidepressant, antidiarrheic, hypolipidic, diuretic and hypoglycemic (Ashraf, 2016). It is recorded in the literature that quince is used in many regions to lower blood sugar (Şenkardeş and Tuzlacı, 2014). In Manisa, Balıkesir, Afyon, Bingöl and Giresun, quince leaves are boiled in water and the tea consumes 1 cup before breakfast in the morning for diabetics in order to reduce blood sugar (Polat et al., 2013; Bulut et al., 2016).

### **3. HYPERTENSION AND HERBAL TREATMENT METHODS**

Hypertension is one of the most common chronic diseases and is a global public health problem, but it is a preventable and treatable disease (Arıcı et al., 2015). Hypertension is responsible for approximately 16.5% of annual deaths worldwide (WHO, 2013) and is the main cause of cardiovascular disease-related morbidity and mortality (Kizhakekuttu et al., 2010). It is estimated that by 2030, the annual death rate from hypertension will reach 23.5 million people (WHO, 2013).

In addition to being an important player at the start of diseases such as hypertension, atherosclerosis, paralysis, peripheral artery disease, heart failure and coronary artery disease, kidney damage can lead to dementia or blindness (Freedman et al., 2016). Hypertension diagnosis is based on increasingly automated blood pressure measurement techniques. For adults over 18 years old, repeated clinical measurements and systolic blood pressure  $\geq 140$  mmHg and/or diastolic blood pressure  $\geq 90$  mmHg are defined as hypertension. Systolic blood pressure is particularly important and is essential for diagnosis in most patients (Wright et al., 2015). The pathophysiology of the essential hypertension depends on the failure of the kidney to secrete sodium at normal blood pressure. The central nervous system, endocrine factors, large arteries and microcirculation also play a role in disorder (Brunstrom and Calberg, 2018).

The use of herbal treatments for cardiovascular diseases treatment and management is increasing. Plants contain a wide range of phytochemicals that have been proven to be protective by reducing the risk of various discomfort and disease (Tachjian et al., 2010).

**Garlic (*Allium sativum*):** Garlic has long been used for a variety of cardiovascular conditions, particularly hyperlipidemia. It has also been reported to have a hypotensive effect. It is thought to increase nitric oxide production, resulting in smooth muscle relaxation and vasodilation. In a meta-analysis study, it was found that garlic reduces blood pressure in patients with increased systolic pressure (Reinhart et al., 2008). While dried garlic is consumed directly for hypertension in Kırklareli region, it is swallowed raw to lower blood pressure in Çerkeş district of Çankırı (Kültür, 2007; Durak et al., 2004). It is recorded in the literature that 1-2 cloves of garlic are directly chewed or swallowed in cases of high blood pressure due to high blood pressure (Polat et al., 2013). It is traditionally consumed raw directly or mixed with yogurt in order to reduce blood pressure in Elazığ (Çakılcıoğlu and Türkoğlu, 2010).

**Hawthorn (*Crataegus monogyna*):** It is a shrub that contains about 300 species and has been used in traditional medicine for the treatment of heart diseases for centuries (Tassel et al., 2010). Pharmacological and clinical studies have shown that it lowers blood pressure. The two main substances that contribute to the beneficial effects of hawthorn on the heart are flavonoids and oligomeric procyanidins, which are powerful antioxidant agents. Rinnophylline, an

alkaloid found in hawthorn, has shown the ability to inhibit platelet aggregation and thrombosis. Thus, it is thought that it may be beneficial in preventing strokes and reducing the risk of heart attack by reducing blood pressure, increasing circulation and preventing plaque formation (Mashour et al., 1998). Fresh flowers and sprouts are boiled in water and consumed in the form of tea by people living around Mount Ergani in Erzincan for hypertension and heart diseases (Korkmaz and Alparslan, 2014). The people of Malatya drink 1 glass of tea on an empty stomach in the morning, which they prepare by keeping the flowers of the plant in hot water for a while for heart ailments (Tetik et al., 2013). In Şanlıurfa, dried hawthorn fruits and leaves are soaked in boiling water and consumed 2-3 glasses a day as a blood pressure reducer (Akan et al., 2005).

**Mistletoe (*Viscum album*):** It belongs to the Loranthaceae family and contains alkaloids, glycosides, phenylpropanoids, tannins, lignin sugars and viscotoxins among various chemical components (Orhan et al., 2005). It also contains various flavonoids such as quercetin, kaempferol and rarely naringenin. Raw mistletoe extract prevents changes in erythrocytes, erythrocyte cell volume, plasma protein levels and erythrocyte sedimentation rate; It has been observed that it prevents changes in blood viscosity, which is an important determinant in the increase of arterial blood pressure (Ofem et al., 2009). Medically, it has been documented as a diuretic, antispasmodic, and beneficial in heart problems (Loeper, 1999). It is popularly used as a remedy for various diseases such as neuralgia, nerve compression, epilepsy, rheumatism



and bronchial asthma, diabetes mellitus, chronic cramps, paralysis, stomach problems, hypertension (Ame et al., 2010; Ohiri et al., 2003). It is recorded in ethnobotanical studies that the branches and leaves of the plant are used for blood pressure lowering in Akseki district of Antalya (Duran, 1998). People living in the Pınarbaşı district of Kayseri, cut the leaves of mistletoe in small and thin ways in case of heart ailments, mix it with rosehip, and drink 1 glass of tea on an empty stomach in the morning together with its branches and trunk (Gençler and Koyuncu, 2005). In Kırklareli region, fruits and leaves are boiled in water by decoction method and filtered, then consumed in 1 glass twice a day for 5-9 days (Kültür, 2007).

**Saffron (*Crocus sativus*):** Saffron is a stemless herb native to Southwest Asia, Spain, Greece, and Morocco and has been used for over 4000 years to treat a variety of medical conditions, including hypertension (Srivastava et al., 2010). Saffron contains several components, including crocin, picrocrocin, safranal and crocetin, flavonoids and anthocyanins, which exhibit different mechanisms of action, including antihypertensive and vasodilator effects. A few experimental studies have shown significant antihypertensive and vasodilatory effects of saffron components (Fatechi et al., 2003) .

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## **CHAPTER 5**

### **MEDICINAL OILS, COMPONENTS AND THEIR USES**

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## **INTRODUCTION**

Thanks to its climate, location, large area, the diversity of plant species grown accordingly, and Türkiye's agricultural potential has become one of the leading countries in the trade of medicinal and aromatic plants. Türkiye's location and importance in the world is arisen because of the fact that many plants grown in Turkish natural area are raw materials used in herbal medicine, plant chemicals, food additives, cosmetics and perfumery industries produced in developed countries. These plants are mostly collected from nature and marketed and they are mainly collected from Aegean, Marmara, Mediterranean, Eastern Black Sea and Southeastern Anatolia Regions. The collected plants are generally thyme, laurel, sage, rosemary, rosehip and linden (Bayram et al., 2010). In order to assess the production and market potential of medicinal and aromatic plants, the products must always be in the desired quality and quantity. Upon increasing importance of medicinal plants in Türkiye, agricultural studies have begun; increasing in breeding studies aimed at cultivating varieties in these plants was observed; varieties conforming the standards have been developed in many medicinal and aromatic plants such as thyme, anise, coriander. Besides fixed oil of the plants, essential oils and macerates obtained from these plants which have been used traditionally for a long time in aromatherapy and phytotherapy in our country which has a very high potential in terms of medicinal plants. Especially St. John's Wort oil, safflower oil, olive oil, black cumin oil, poppy oil, lavender oil, thyme oil, bay oil, sage oil kinds are included in well-known and commonly

used oils. Medicinal oils are sold in herbalists, herbal product stores, pharmacies and some markets in our country.

There is still no detailed research on medicinal oils and their uses in our country, and what their standards will be, by whom they will be produced, where they will be sold, and by which ministry they will be followed. Such deficiencies should be identified and concern standards should be composed. It is also necessary to examine and determine the quality parameters of medicinal oils. Where the raw materials of medicinal oils are obtained from; whether the agriculture is realized by the manufacturer or not; in another mean their origin should be known.

While extracting oils, it is necessary to pay attention to some issues as follows:

- Fixed oils, essential oils, hydrosols, oily macerates, etc. must certainly be obtained from reliable sources.
- The production permits and expiry date of the products that will be purchased must be certainly checked.
- Products of reliable and well-known companies must be preferred.
- It should be ensured that the herbal drugs used in the oil production are correct, and it should be paid attention to ensure that they are clean and free of foreign bodies. If it is possible, products produced from drugs obtained from certified production sites should be preferred.
- Since all products on the market are not in the same quality so brands should be researched very well.

- It should be considered that the products may cause skin disorders such as allergies, itching and redness. Before using the product, it should be tested with a patch test on a very small skin surface.
- Pure essential oils should not be applied directly on the skin. It must certainly be diluted with a carrier oil.
- Obtained herbal medicinal oils, hydrosols, oily macerates and other products must be tightly closed, stored in amber which is dark colored bottles, and stored in a cool, dry and shaded place in name of not to deteriorate quickly and react.

In this study, the most well-known fixed oils and essential oils, which are widely used internally and externally in our country, are mentioned.

### **1. CLASSIFICATION OF MEDICINAL OILS**

Many oils, macerates, hydrosols and their derivatives are used under the name of medicinal oils, which are used among the people. The most well-known groups are essential oils and fixed oils. The classification of medicinal oils is as follows;

1. Essential Oils (Ethereic oils, essences)
2. Fixed Oils
3. Oily Macerates
4. Resin and Balms
5. Concretes, absolutes and enfleurages
6. Hydrolats (Hydrosols, aromatic waters)
7. Ozonated fixed oils

Essential oils are volatile, strong-smelling and oil-like volatile substances obtained through water vapor, water-steam or direct steam distillation from herbal drugs and do not leave any stains when dropped. Sage, lavender, peppermint, eucalyptus, oregano oils are examples of essential oils. Fixed oils are substances obtained from the seeds of plants through cold pressing method or solvent extraction, which do not disappear when dropped. Hydrolats are also known as aqueous solutions and are the aromatic waters remaining under the oil while obtaining essential oil from essential oil plants. Anise, thyme, peppermint, laurel and rose are the best known hydrolats. Fixed oils are used as carriers in the preparation of mixtures containing essential oils. Examples of fixed oils are linseed, sesame, poppy, mustard, jojoba oil. Ozonated Oil is obtained through injecting ozone gas into oil. Especially olive oil and St. John's Wort oil are ozonated. Oily macerates are oily mixtures prepared through keeping herbal herbs and drugs as whole, crushed or grinded in fixed oil for a certain period. The soaking process is carried out in the sun and/or shade, depending on the solubility of the active ingredient of the oil that will be prepared. Macerates are not the oil of the plants themselves, but they are mixtures carrying the components of the plant after the oil-soluble active substance passes into the oil. Centaury oil can be given as well-known example. In addition, calendula oil, rosemary oil, marshmallow oil, comfrey oil can be given as other examples (Demirezer et al., 2021).

Resin is a solid or semi-fluid, hard to crystallize, insoluble in water, soluble in organic solvents, softening and melting when they are

heated. Resins are found in plants either dissolved in an oil or together with gums. They are often found with essential oils (oleoresin), gums (gummirezin), or with essential oil and gum (oleogummirezin).

There are many resin-bearing plants in plant families such as pine, legumes, parsley (Anonymous, 2022). Examples are salai (*Boswellia serrata*), frankincense, Pistachio (Pistachio Gum), Pine, Laden, *Pistacia terebinthus*, Gum tree (Gum Drop). On the other hand, Balsam is a grayish resin structure containing benzoic, cinnamic acids and esters of these acids (Demirezer et al., 2021).

Fresh plant material is extracted with a suitable nonpolar organic solvent (such as benzene, hexane, heptane) and then the essential oil mixture, fixed oil, colorants and waxy substances obtained as a result of evaporation of the organic solvent at low pressure is called 'concrete'. The best example of concrete is the rose concrete. Absolve is obtained as a result of extraction of concrete with ethanol. Ethanol is removed by evaporation. Rose, water lily, violet are examples of absolve. Enfleurage is a method used since ancient times to obtain essential oils, especially from delicate flowers and flower petals with very trace amounts of essential oil. It is a product formed by spreading the flowers on an odorless fixed oil applied on a thin glass plate and waiting for a few hours, then extracting the oily mixture with aqueous ethanol and removing the solvent. There are two methods as cold and hot enfleurage. The lilac enfleurage can be given as an example. (Demirezer et al., 2021; Anonymous, 2022).

## 2. ESSENTIAL OILS

Essential oils are volatile fragrant components which do not leave any traces in the environment, mostly obtained from aromatic plants by water, water vapor or steam distillation. Most of the essential oil are very valuable because they are obtained from very high amounts of herbal drugs. Especially Isparta rose essential oil, lemon balm (Oltograss) essential oil, medicinal chamomile essential oils are very valuable. In addition, essential oils are diluted 1-3% according to the intended use. Essential oils are not mostly used internally. Also, they are not applied directly to the skin (Demirezer et al., 2021).

The most well-known essential oils by the public are as follows;

- 2.1. Laurel essential oil
- 2.2. Lavender and Lavandin essential oil
- 2.3. Isparta Rose essential oil
- 2.4. Rosemary essential oil
- 2.5. Peppermint (Medical Peppermint) essential oil
- 2.6. Melissa (whitey wood (melicytus ramiflorus) essential oil
- 2.7. Medicinal Sage essential oil
- 2.8. Lemon Verbana (lemon beebrush) essential oil
- 2.9. Thyme essential oil.

### 2.1. Laurel Essential Oil

It is *Laurus nobilis* L in Latin. It is obtained by distillation of water from the leaves of the laurel tree. It is known as *Laurel leaf* or *Bay Laurel* essential oil in English. It is  $\alpha$ -pinene,  $\beta$ -pinene, 1,8-cineol, limonene. It is widely used in rheumatic diseases, amenorrhea, colds

and flu, throat infections. It may cause allergies (Demirezer et al., 2021; Lawless, 2013; Gruenwald et al., 2004; Aromaweb, 2022a).

## **2.2. Lavender and Lavender Essential Oil**

When it is said Lavender Essential Oil, the essential oil obtained from the *Lavandula angustifolia* Mill specie should come to mind first. Its other name is *British Lavender*. Another name of Lavandin essential oil is *French Lavender* essential oil. Lavandin oil is obtained from the species of *Lavandula\*intermedia* Emeric ex Loisel. This species emerged from the hybridization of *Lavandula angustifolia* with *Lavandula latifolia*. Both types have the same usage. However, lavandin essential oil contains a higher rate of camphor (camphor) as a component. Other main components are linalool and linalyl acetate. It is effective in diseases as: upper respiratory tract infections, muscle pain, migraine, insomnia, relieving stress and mental fatigue (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

## **2.3. Isparta Rose Essential Oil**

It is also known as *rose oil*. It is grown intensively in Isparta, in our country. It is *Rosa damascena* in Latin. The main components of rose oil are citronellol, geraniol, nerol, neral. It is used in many ways to revitalize the skin, to equalize the skin tone in redness, to prevent wrinkles, to prevent dryness of the scalp and dandruff and to moisturize it in cosmetics; also, it is used to relieve headaches thanks to its refreshing-cooling effect, in fever diseases, as a comforter when



smelled (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

#### **2.4. Rosemary Essential Oil**

It is *Rosmarinus officinalis* L. in Latin. Its essential oil is obtained from the green and thin leaves of rosemary. The main components of the essential oil are: 1-8 cineol,  $\alpha$ -pinene, camphor, and the others are camphene, borneol, bornyl acetate,  $\beta$ -caryophyllene, p-cymene, limonene, linalool, myrcene,  $\alpha$ -terpineol, verbenone. It is used in indigestion, as a relief in the digestive system, in muscle and joint pains, in circulatory problems such as increased blood pressure, in rheumatic diseases, in headaches, as an antimicrobial with the help of a diffuser in the environment (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

#### **2.5. Peppermint (Medical Peppermint) Essential Oil**

It is *Mentha piperita* L. in Latin. It is known as peppermint. Its main components are menthol (35-45%), menthone (15-20%), methyl acetate (3-5%), neomenthol (2.5-3.5%), isomenthone (2-3%), limonene, and also pulegone. Medicinal peppermint oil should not be confused with spearmint (*Mentha spicata*) and Japanese Peppermint (*Mentha arvensis*). The type of peppermint oil should be stated on the label. Although medical mint has many usage fields, it is most commonly used in migraine and headache diseases. It is widely preferred for digestive relief, pain reliever, cough suppressant, regional muscle pain. It is applied internally in the form of capsules and chewable tablets in gastrointestinal disorders; 1-2 drops are massaged

to the temporal region with fingertips in migraine disease; 2-3 drops of peppermint oil are dropped into a carrier oil (olive oil) in muscle pain and itchy skin disorders, or applied with the help of cotton. In cold and cough diseases, it is applied by inhalation, in another mean, in the form of inhalation, through steaming - adding a few drops into boiling water (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

### **2.6. Melissa (Lemon Balm) Essential Oil**

Melissa essential oil is often confused with the lemon beebrush essential oil (*Aloysia citrodora-Lippia citriodora*). While buying essential oil and in this point it should be specified as lemon balm and the label should be paid attention. Its Latin name is *Melissa officinalis* L. It is too valuable because of its obtaining essential oil trace amounts. An oil in the range of 0.06-0.8% is obtained from 100 g of the drug. Its main components are geranial, neral, citronellal, linalool, geraniol, geranylacetate, methyl citronellate. It is used as a sedative and relaxing in sleep problems, nervous diseases, depression, menstrual pain problem; it is used through inhalation in nervous-related heart and circulation problems. Inhaling lemon balm oil before going to bed facilitates the transition to sleep especially in insomnia problems (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

## 2.7. Medical Sage Essential Oil

Essential oil obtained from medicinal sage should not be confused with essential oils obtained from species known as clary sage (*Salvia sclerea*), Anatolian sage (*Salvia triloba*) and Mountain sage (*Sideritis* sp.). It is in *Salvia officinalis* L. Latin. Alpha-thujone, beta-thujone (20-60%), 1,8-cineole (6-16%), camphor (14-37%), borneol, isobutyl acetate, camphene, linalool, alpha and beta-pinene, viridiflorol, Alpha and beta-caryophyllene (humulene) are the main components of sage essential oil. Medicinal sage herb is consumed in upper respiratory tract infections, especially in throat infections. In the same way, the essential oil can be applied as gargle through dropping a few drops into the water gargle for mouth and throat infections. The oil is not used directly, but mixed with a carrier oil and applied with cotton in some skin disorders. It is also applied in the form of vapping for throat infections (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

## 2.8. Lemon Verbana (lemon beebrush) Essential Oil

It is *Lippia citriodora* (*Aloysia citrodora* – *Aloysia triphylla*) in Latin. It should not be confused with lemon beebrush essential oil. This plant is known as lemongrass. Geraniol, neral, nerol are its main components. It is used as an antiseptic, antispasmodic, carminative, sedative, ataraxic (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

## 2.9. Thyme Essential Oil

*Thymus*, *Origanum*, *Thymbra*, *Satureja* and *Coridothymus* species naturally found in the Turkey flora are known and consumed under the name of thyme. Essential oil obtained from these 5 genera species are sold as 'thyme oil' among the public. However, certain companies produce thyme oil from species included in *Thymus* and *Origanum* genera in our country. Essential oils obtained from *Thymus vulgaris* and *Origanum vulgare* and *Origanum majorana* species are used. The thyme oil is consumed directly through dripping 2-3 drops into sugar cubes for upper respiratory tract infections and stomach disorders among the people. It is also applied in aromatherapy, in lotions and ointments form due to its antiseptic effect, in mouthwashes, it is used in the massage form for muscle and rheumatic pains (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022a).

## 3. FIXED OIL

They are substances that leave traces in the environment and obtained by cold pressing or extraction from the fruits and / or seeds of medicinal and aromatic plants. They are also important because of their composing major fatty acids. Olive oil, sunflower oil, corn oil, safflower oil, black cumin oil, linseed oil, grape seed oil can be given as examples. Most of the fixed oils are used as carrier oil in the preparation of essential oil mixtures, and most of them are also consumed alone, such as black cumin oil. These oils are mostly

obtained by cold pressing in order not to damage their components and fatty acids in their chemical structures.

Fixed oils having the highest consumption are as follows;

- 3.1. Black cumin oil
- 3.2. Flaxseed oil
- 3.3. Sesame Oil
- 3.4. Grape Seed oil
- 3.5. Poppy oil
- 3.6. Safflower oil
- 3.7. Olive oil

### **3.1. Black Cumin Seed Oil**

Black cumin seed oil is obtained by cold pressing (cold press) method and is sold in herbalists' shops. Black cumin is known as *Nigella sativa* L. in Latin. It is 'Black Cumin Seed Oil' in English. The oil is extracted from the seeds of this plant through cold pressing method. Its shelf life is approximately 2 years in the dark. It is mostly consumed directly in asthma, relief of allergic disorders, diabetes, cough and digestive disorders. Thanks to its immune system strengthening feature, it increases body resistance. Studies are continuing to show that it has a lowering effect on bad cholesterol. It is applied externally in rheumatic diseases, as nourishing hair, and in psoriasis diseases. Black seed oil is also consumed directly or in capsule form. Recently, there are studies showing that using it on fasting is also supportive in losing weight (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022b).

### **3.2. Flaxseed Oil**

It is *Linum usitatissimum* L. in Latin. Linseed oil is also obtained by cold pressing method. If heat treatment is applied in the production of linseed oil, linseed oil is formed and it is used in paint thinner and varnish production in this industry, it is not suitable for consumption as food. Therefore, if it is to be consumed as food, the cold press method must be preferred. Flaxseed and its oil are used by dieters to prevent constipation and to accelerate metabolic rate. It is one of the oils having the highest omega-3 fatty acid and it is said it has effect for lowering cholesterol and high blood pressure. The flaxseed is mostly included in skin softening, moisturizing and soothing cream formulations. It is also applied directly in hair care due to its moisturizing and nourishing features of the hair (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021).

### **3.3. Sesame Oil**

The sesame plant is *Sesamum indicum* L. in Latin. Sesame oil is obtained through cold pressing method. It is applied in cream form or directly due to its effects against hair loss, softening the skin, increasing its elasticity, revitalizing and reducing blemishes in cosmetics. It can be said that it is also indirectly effective against skin aging. Sesame oil can be used as food in salads. It is said to have a cholesterol-lowering effect in direct use. While it is mostly preferred in meals and salads as a flavor enhancer in China, India and other Far East Countries, it is mostly preferred in hair care and skin creams in our country (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022b).

### 3.4. Grape Seed Oil

It is a fixed oil obtained through cold pressing method from the seeds of the grape fruit. The grape is *Vitis vinifera* L. Latin. Grape seed oil has been widely preferred in recent years, especially since it helps to protect against cardiovascular diseases. The resveratrol component in grapes is found in the skin and seeds. Due to the presence of this component in the kernel, grape seed oil has a strong antioxidant effect. There are also studies on its effect for lowering bad cholesterol. It is also mentioned with its blood cleansing and cell regenerative properties. It is said that it has effective in the disappearance of acne and blemishes on the skin, thanks to its cell regenerating and moisturizing properties in the skin (Gruenwald et al., 2004; Lawless, 2013; Güçlü, 2019; Demirezer et al., 2021; Aromaweb, 2022b).

### 3.5. Poppy Oil

Poppy oil is a fixed oil obtained from the seeds of the poppy plant through cold pressing method. It is *Papaver somniferum* L. in Latin. The poppy (*Papaver somniferum* L.) plant has two important products; one is the capsules and the alkaloids in the capsule, the other is its seeds and oil. In other words, poppy is important oil plant in terms of both a medicinal and industrial area. The importance of poppy seeds and oil is clearly stated in the Bible and especially in the Talmud. The most grown poppy varieties in Turkey are respectively blue, white and yellow seeds. Beside the oil in the seed is used for edible purpose, it is used in paint, varnish industry, soap making in other industry branches because of its being a semi-drying oil. Poppy oil is rich in Omega-6 and Omega-9

fatty acids. This oil contains high levels of vitamin E. It has antioxidant effect. There are records that it is used in goiter disease in Latin America due to its high iodine content. It helps your skin to appear younger and regulate the functions of all skin cells through preserving the skin moisture. It is used as massage oil in aromatherapy (Gruenwald et al., 2004; İpek and Arslan, 2012; Lawless, 2013; Demirezer et al., 2021).

### **3.6. Safflower Oil**

Safflower is a plant that has become popular in our country in recent years. It is *Carthamus tinctorius* L. in Latin. Its oil is used both for cooking and in the production of biodiesel. It is commercially sold as 'false saffron' because its flowers resemble the saffron plant. There are thorny and thornless safflower types. The amount of oil in thornless safflower species is higher (approximately 30-40%). Safflower oil is rich in oleic and linoleic fatty acids. In this regard, it has started to be produced by some oil companies in our country as edible oil in recent years. The encapsulated form is recommended by dietitians because safflower oil has a losing weight effect. It is effective in asthma and cold. The encapsulated form of Safflower oil is allowed to be used for control purposes in diabetes like black cumin oil. In addition, it revitalizes the skin structure, strengthens the hair follicles, makes it grow faster and looks livelier (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Öztürk, 2022).



### 3.7. Olive Oil

Olive oil is a fixed oil obtained from olive fruits. It is *Olea europaea* L. in Latin. Olive oil is classified as extra virgin olive oil, refined olive oil, riviera olive oil, refined pomace oil, mixed olive oil. It is widely consumed as edible oil in Mediterranean countries. The main unsaturated fatty acids in olive oil are; oleic, linoleic and linolenic acids. Oleic acid is the major fatty acid of olive oil and composes its 55-85%. It is the most recommended oil for cardiovascular diseases. It has a cholesterol lowering effect. It has the effects of accelerating metabolic rate and regulating digestion. It is traditionally, commonly preferred for digestive problems in infants and children. In addition, olive oil is the most preferred oil for dilution of essential oils and ozonation method. When ozone oil is mentioned, the ozonated form of olive oil comes to mind first. It has a refreshing and moisturizing feature for skin therefore there are many moisturizing and refreshing creams with olive oil. On the other hand, it is preferred to apply directly or in products such as serum and shampoo as a skin revitalizer, anti-shedding agent and moisturizer in dry hair (Gruenwald et al., 2004; Lawless, 2013; Demirezer et al., 2021; Aromaweb, 2022b).

### 4. OILY MACERATS

The composition obtained through transferred of the oil-soluble components of the plant into the fixed oil is called *macerate oil* or *oily macerate* as a result of keeping certain plant parts of medicinal and aromatic plants in a suitable fixed oil for a certain period of time. The best examples of macerate oils are St. John's Wort oil, calendula oil and

Bitter gourd oil. There are also macerates of aloe, arnica, rosemary, yarrow, chamomile, gotu kola, marshmallow, carrot, lavender, comfrey, licorice, saffron, garlic, medicinal chamomile.

#### **4.1. St. John's Wort Oil**

The St. John's Wort is called as *Hypericum perforatum L* in Latin. It is known as St. John's Wort Oil in English. It is obtained through placing the harvested fresh herbs of St. St. John's Wort during the full flowering period, in extra virgin olive oil and keeping them for a certain period of time. Approximately 350-500 g of fresh herbs are placed in a transparent glass jar, 1-1.5 liters of olive oil is added, and left under the sun for 4-6 weeks. At the end of the period, the oil is filtered and St. John's Wort oil is obtained. St. John's Wort flowers, which leave a red color on the hands when touched with bare hands, turn red at the end of the maceration, as these components are dissolved in olive oil. St. John's Wort oil is used through applying directly on eczema, skin burns, skin wounds, 1<sup>st</sup> degree burns and bedsore. It is also included in diaper rash creams for babies. It is also recommended to take orally for stomach and digestive disorders. It is used through direct application in muscle pain, rheumatic diseases and other pains. Finally, St. John's Wort oil is also prepared by ozonation. It is known as ozonated St. John's Wort oil.

#### **4.2. Calendula Oil**

Calendula is known as medicinal calendula. It is *Calendula officinalis L.* in Latin; is known as Calendula oil, Marigold oil. This oil is obtained by collecting the yellow-orange flowers of the plant and keeping them in a fixed oil like mostly olive oil for a certain period of

time. The flowers are kept in oil as fresh or dried. It is kept in the sun for about 15-45 days. At the end of the period, after filtration calendula oil is obtained. Calendula oil is mostly used in cosmetics field. It repairs the skin and strengthens the barrier function of the skin. It helps preventing skin dryness. It is found in sunscreens and sunburns and even in baby diaper rash creams. Instead of direct use, it is applied to the concern area as an ointment with a certain amount of Vaseline or vegetable fat (1:5 or 1:25 ratio) or olive oil (1:10).

#### **4.3. Balsam Apple, Bitter Gourd (*fructus momordicae*) Oil**

The Latin for the bitter gourd plant is *Momordica charantia* L. and its oil is known as Bitter melon oil, Bitter gourd oil. The ripened fruits of the plant are kept in olive oil or sweet almond oil for 3-6 months to make a paste consistency. This prepared paste-like oily mixture is used internally and externally. Unlike the others, it is obtained without filtering. The bitter gourd oil is consumed internally twice a day on an empty stomach for the healing of stomach ulcers. Maserate is applied directly for skin disorders such as wounds, burns, eczema and psoriasis. It has been stated in studies that the bitter gourd oil should not be used especially in allergic patients.

### **5. CONCLUSION**

Oils obtained from medicinal and aromatic plants have been used for many years for different purposes, mainly cosmetics, medicine, food, industry, aromatherapy and phytotherapy. Especially fixed and essential oils have attracted attention due to their wide range of uses, and their chemical structures and their biological activities have been

the subject of curiosity. In this review study, brief information has been given about fixed, essential oils and oily macerates, which are widely used by the public in our country.

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## **CHAPTER 6**

### **WEED MANAGEMENT IN MEDICINAL AND AROMATIC PLANTS**

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## **INTRODUCTION**

Weeds are one of the main problems in medicinal and aromatic plants (Upadhyay et al., 2012; Hendawy et al., 2019). Knowing the ecology and biology of the weeds seen in the areas where these cultivated plants are cultivated is essential for identifying these weeds (Pala & Mennan, 2022). Depending on the type of product produced, annual, biennial, and perennial weeds also differ according to their class with grass and broadleaf weeds (Blanco, 2016). This difference also causes diversity in the method of management to be chosen. In addition to identifying weed species, weed interaction and competition with the cultivated plant also closely depends on weed density and frequency of encounter. According to this prevalence (frequency), weeds cause quality and yield loss at different rates in the product (Oerke, 2006). Cultural measures and mechanical and chemical tactics for controlling weeds are often preferred. Now let us examine weed management in medicinal and aromatic plants. For weed control in plants with relatively wide inter-row and over-row distances, agricultural workers can be weeded by manual picking and hoeing. Also, tillage tools such as rakes, cultivators, and plows that are attached to the tractor and move from the tail shaft can be used (Shamkuwar et al., 2019). Unlike other industrial field crops, chemical control is not recommended in the fields of medicinal and aromatic plants (Pala and Mennan, 2022). The reason for this is the limited growing areas, the inadequacy of licensed herbicides, and the concern that the chemical used can affect the aroma and medicinal properties by participating in the structure of the plant. Since it is practical and economical, herbicides, which are widely used



in other products, and are not used in medicinal and aromatic plants, make the weed problem in these products even more troublesome. There is a need to develop alternative weed control methods for these products, whose crop areas are increasing yearly (Upadhyay et al., 2012). For example, herbicide licensing can be achieved in these products (primarily allelochemicals or biochemicals). Also, preventive measures such as quarantine measures and the use of certified seeds, cultural measures such as mulching and solarization, mechanical techniques such as thermal and robotics, and biological methods such as microorganisms and insect use can be integrated into existing practices (Fennimore et al., 2016; Junior et al., 2022; Maurya et al., 2022). In recent years, it has been seen that research on allelopathy in weed management in medicinal and aromatic plants has increased (Islam et al., 2018; Li et al., 2019).

Medicinal and aromatic plants are products of a particular plant, such as leaves and flowers (Ozyazici, 2017; Ozyazici and Kevseroglu, 2019; Bicer and Ozyazici, 2020). To maintain the quality and obtain high yields in these parts, which have commercial value, it should be ensured that the growing area is weed-free during critical periods such as germination development, flowering, and seed binding (Sahu, 1984). Therefore, it is necessary to decide on weed control according to the threshold damage. In addition, it is crucial to choose appropriate tools and machines for weed control in plants with different agricultural demands and care. Weeds make harvesting difficult and can mix with the harvested product and affect the metabolites of the product with

aromatic and medicinal properties (Upadhyay et al., 2012; Hendawy et al., 2019). As it is understood, weeds should be tried to be kept under pressure by determining the weeds that are primarily problematic in medicinal and aromatic plants. Determining their density, frequency of encounters, and coating area-preferring appropriate non-chemical weed control methods (such as cultural, physical, and biological) according to the critical period and economic damage threshold.

### **1. AN OVERVIEW OF WEED MANAGEMENT IN MEDICINAL AND AROMATIC PLANTS**

When we look at the definition of a weed, "unwanted plants in a particular place" are called weeds (Pala and Mennan, 2022; Shrestha et al., 2022). On the other hand, wild plants refer to plants that grow spontaneously in nature. Medicinal and aromatic plants also intersect with weeds and wild plants. Plants used in treating human and animal diseases are called medicinal plants, and fragrant plants are called aromatic plants (Giannenas, 2020; Ozyazici, 2020) (Figure 1). In the world and our country, medicinal and aromatic plants are increasing daily in the pharmaceutical, cosmetic, food, and feed industries (Fierascu, 2021; Ozyazici, 2021; Tasci, 2022). Apart from the medicinal and aromatic plants produced, all the plants in the growing area are seen as weeds and tried to be controlled. Prevention, control, eradication, and management procedures are carried out to manage weeds seen in medicinal and aromatic plants (Mirmostafae et al., 2020). The most challenging part of weed management in such sensitive

crop plants is the inability to prevent the contamination of their growing areas by weeds and avoid using chemicals in weedy areas.



**Figure 1.** Weeds in the field of medicinal and aromatic plants

Prevention addresses a potential problem that does not yet exist. The results of preventive efforts are more challenging to observe and measure. It is difficult to show that a weed problem does not arise due to weed prevention. As preventive measures, quarantine includes using certified seeds, clean equipment, burnt animal manure, and clean animal feed (Peerzada et al., 2019; Junior et al., 2022). The aim is to prevent the spread of weed seeds and other vegetative materials. It covers diagnosing weeds and treatments to find them before they become a problem and prevent them from spreading. Weed control involves many techniques used to limit weed infestation and minimize

competition. These techniques try to strike a balance between the cost of control and crop yield loss, but weed control is used only after the problem has arisen; that is, it is not prevention. The problem is known and visible, and the actions can be adapted to the practical problem. Control techniques can be selected to meet short-term economic and agricultural planning goals.

Weeding is the complete control of vegetative reproduction sections such as rhizomes, stolons, onions, tubers, and weed seeds by complete elimination, i.e., 100% elimination (de Aguiar et al., 2022). This easy-sounding practice is exceedingly difficult to achieve, and eradication efforts are rarely entirely successful. Germinated weed plants with herbicide application and mechanical techniques are typically easy to control because they can be seen, and the control techniques are effective. Seed and vegetative reproduction parts are difficult to destroy in the soil since they are not seen and appear in one season. Dormancy allows these reproductive parts to emerge over the years (Batlla et al., 2020). Eradication is the best program for small populations of perennial weeds, but current technology has not been able to facilitate this. In weed science, prevention is better than control, but control should be carried out when necessary because weeds are transmitted unannounced or have already been in the soil. Prevention and destruction require long-term thinking and planning. Techniques such as mulching and solarization can be applied practically for weed control in medicinal and aromatic plant areas (Figure 2).



**Figure 2.** Mulching

Weeding in medicinal and aromatic plants is a combination of prevention, control, and eradication techniques to manage weeds that are a cultivated plant, farming system, and problem (Abouziena et al., 2016). Producers/farmers need to consider crop history, alternation status, weed contamination, and control methods cost before making appropriate management decisions (Liebman and Dyck, 1993). Keeping the populations of weeds, which are problematic in weed management in medicinal and aromatic plants, at a low level may be the most easily achieved, financially wise, and environmentally desirable goal.

Weed control can cure a weed infestation but does not entirely prevent it (Strehlow et al., 2020). Knowing weed seedlings and mature

plants is the first step in developing a plan and management program for each crop and field. Since there are limited control methods in medicinal and aromatic plants, it is always aimed to be alert to new weeds that may be a problem and to prevent them from controlling as much as possible (Ekwealor et al., 2019). Various preventive practices can be included in management programs. These include isolation of farm animals, use of clean farm equipment and cleaning of mobile equipment, including harvesters, using certified production material, development of quarantine measures to prevent invasive species, and regularly visiting fields to identify new weeds. The application of eradication in the first transmission of new species, the removal of weeds in the fields and channels and the prevention of contamination, the preference of modern irrigation methods such as drip irrigation because irrigation with streams and streams increases weed seed contamination, the collection, mowing or control of weeds before seed yielding (Liebman and Janke, 1990; Harker and O'Donovan, 2013). In weed management in medicinal and aromatic plants, manual picking, sorting, mowing, or hoeing with humans is not preferred unless it is mandatory due to both the supply of workers and the fact that it is expensive (Figure 3).

It is known that chemical methods do not develop in medicinal and aromatic plants (Carrubba and Militello, 2013). In biological management, we are still at the beginning of the road. Unlike the methods listed above, mechanical weed control is widely used. While the weeds between the rows can be well controlled with mechanical

management, controlling the weeds on the row is not at the desired level. For this purpose, in recent years, it has been aimed to increase the control rate on the weeds and the weeds between them with sensitive sensor sensors, intelligent cultivators, and robotic approaches (Fennimore et al., 2016) (Figure 4).



**Figure 3.** Hoeing (HGIC, 2022)





**Figure 4.** Robotic weed control (Fennimore et al., 2016)

Mechanical weed control can be more expensive due to the time and cost of equipment and fuel required. Success almost always requires several trips to the site, precise timing, and favorable weather conditions. In addition, the farmer should have more information about grass and crops. Finally, successful mechanical control requires careful planning to get the timing and tool right.

## **2. APPROACHES TO WEED PROBLEMS IN SOME MEDICINAL AND AROMATIC PLANTS**

The primary reason for the preference is that the area selected for medicinal and aromatic production is weed-free (Carrubba et al., 2015). Therefore, before winter planting, if possible, planting should be delayed, and after the first rains, weeds should be removed by plowing the soil (Figure 5). In summer plantings, this process can be carried out during the spring rains.





**Figure 5.** Tillage (UGA, 2022)

If there are weeds around the farming area, that is, at the edge of the field, they should be plowed or mowed before tilling the seed, and in acute cases, they can be dried with total herbicides. Controlled partial incineration can be done in plants that are difficult to control, such as common reed, in local areas. Filters can be used to prevent the contamination of weeds from irrigation canals. If possible, sprinkler or drip irrigation methods should be preferred instead of keel irrigation. If animal manure is used, care should be taken to ensure that weed seeds are burned to prevent contamination. Certified seed should be used to prevent the contamination of weed seeds in the growing area. The tools and machines used for planting, maintenance, and harvesting should be disinfected with substances such as hypochlorite acid, washed with

plenty of water, and then brought from one field to another. To prevent transmission with animals, herds should not be introduced into the growing area. In short, the farming area should be kept weed-free. However, since it is generally known that thousands of seeds can be dormant in one cubic meter of soil and germinate at regular intervals, it is vital to keep records of these weeds (Baskin and Baskin, 1998; Neve et al., 2009). Prevention of germination of weeds, early control after germination, or pre-seed binding control is essential. When caring for medicinal and aromatic plants, the weed's biology and ecology should be considered, and the area's density should be tried to be reduced (Carrubba et al., 2015). Weeds seen in the growing area need to be removed.

Weeds have a high ability to adapt, compete, reproduce, and spread due to their native soil offspring or invasive properties (Bhowmik, 1997). Changes in weed records should be recorded. It should be determined whether the newly infected species are invasive or not. Hazard situations should be determined according to weed species' effect on the product's metabolites. Under stress conditions caused by weeded areas, deterioration may occur in the metabolites to be synthesized by the products (Upadhyay et al., 2012). The weed problem that causes stress should be eliminated to prevent this situation. The response to weeds varies significantly according to plant species, density, frequency of encounter, and metabolites depending on the coverage area. There is a need for research on the effect of weeds on the metabolites of medicinal and aromatic plants.

There is competition between medicinal-aromatic plants and weeds for access to light, water, and mineral substances (Upadhyay et al., 2012). The product's competitiveness decreases due to the high density of weeds in early planting. On the other hand, when frequent planting is done, the germination and development of weeds are suppressed because the number of cultivated plants will be high in the unit area (Islam et al., 2006). The growing environment of plants affects vegetative/generative development and weed competition. For example, common garden sage' plants are smaller at height, have fewer leaves per plant, and reduced leaf size (Bezzi et al., 1992; Karamanos, 2000). Wormwood has seen plant height increase with sparse planting (Maw et al., 1985); shatavari provides a higher fresh weight and root count per plant over large ranges (Tewari & Misra, 1996). It has been determined that common fennel exhibits more branching when the row interval increases and blooms more per plant (Al-Dalain et al., 2012). From this, it can be concluded that when weeds are controlled, if there is a sufficient level of water and nutrients in the soil, there is an increase in the number of plants, biomass, and yield per unit area.

Plants tend to grow taller and be able to reach sunlight. Although there was a weak development in the early periods, the prolongation of wormwood increased (Maw et al., 985). Harvest time should be carried out when the weed is weak and the plant's high competitiveness. The optimum plant level at harvest maximizes biomass/seed yield, as in the case of black cumin (Moghaddam and Motlagh, 2007) or spice cumin (Shakeri et al., 2015) species, where seed production decreases as the

number of plants increases (Falzari et al., 2006; Shakeri et al., 2015). A decrease in the yield of essential oil of thyme has been detected due to weed and other adverse environmental factors in which weed competition with cultivated plants influences the production and composition of secondary metabolites. Increased plant biomass (in areas outside where metabolites such as twigs and stems are collected) does not always mean an increase in essential oil content (Scarpa et al., 2004).

In order to identify weeds that are problematic in medicinal and aromatic plant species and their growing areas and accordingly to ensure that the competition between the crop and weed is in favor of the product since scientific research is needed to determine the appropriate ones for the cultivation norm and other agricultural processes (Islam et al., 2006; Upadhyay et al., 2012). Studies on weeds, which are a problem in medicinal and aromatic plants, are insufficient. These weeds vary according to the medicinal and aromatic plant grown. Common weeds are *Alopecurus myosuroides*, *Amaranthus retroflexus*, *Avena sterilis*, *Bromus tectorum*, *Chenopodium album*, *Cirsium arvense*, *Convolvulus arvensis*, *Cuscuta* spp., *Cynodon dactylon*, *Cyperus rotundus*, *Lolium rigidum*, *Orobancha* spp., *Phragmites australis*, *Plantago lanceolata*, *Polygonum aviculare*, *Poa annua*, *Prosopis farcta*, *Rumex crispus*, *Sinapis arvensis*, *Sorghum halepense* are known to be such species. Most of the weeds that are the problem appear to be annual. If it is a cultivated plant, it can be cropped (Martínez-Ghersa et al., 2000; TOB, 2022).

Weeds are known to cause economic losses in the used parts of medicinal and aromatic plants (Lubbe and Verpoorte, 2011). For example, in the herb of sage and chicory; on the leaves of mint, sage, and lemon balm; in the flower of chamomile and marshmallow plant; in the fruit of rosehip, anise, and coriander; in the seed of flax and fenugreek; at the root of valerian and licorice; in the rhizome of the discrete grass; in the tuber of the salep plant; causes yield and quality loss in the onion of the garlic plant.

The yield and quality of medicinal and aromatic plants are inversely proportional to the incidence of weeds in growing areas (Dajic-Stevanovic and Pljevljakusic, 2015). For example, 34% in sweat (Shehzad et al., 2011), 90% in coriander and fennel (Shehzad et al., 2011; Carrubba and Militello, 2013), 51-64% in saffron (Norouzzadeh et al., 2007), 20% in hyacinth (Bonasia et al., 2012), 75% in sage (Satvati Niri et al., 2015).

The removal of weeds and the reduction of their biomass in growing areas cause the plant to develop vegetatively, as in the case of cress, to make the plants taller, increase the number of branches and increase the number of seeds per plant (Shehzad et al., 2011; Nadeem et al., 2013; Sayyidi et al., 2016). However, it should be noted that reducing the total weed biomass is not the only factor affecting yield.

It should not be forgotten that the growing area in the fields to be planted may be exposed to weed infestation (Ziska et al., 2011). Therefore, Weedweeding control is especially critical in the initial period (first six weeks) in species such as thyme (Zumelzù et al., 1999),

sage (Karamanos, 2000), fennel (Yousefi and Rahimi, 2014), and coriander (Diederichsen, 1996), which are slow to develop in the initial period.

Medicinal and aromatic plants can be affected by weeds in many ways (Zhang et al., 2010). Weeds adversely affect the product's commercial value both by competition and by mixing with the product (Upadhyay et al., 2012). Even if uncontrolled weeds are subsequently controlled during critical periods, there is a decrease in the content of metabolites, such as in coriander and milk thistle (Gil et al., 1998; Kothari et al., 2002; Sarrou et al., 2016; Zheljzakov et al., 2006).

Removal of weeds cumin, musk sage, coriander, chamomile, mint, thistle "Mary thorn," fennel, *sage*, marjoram "Istanbul thyme," and thyme has been found helpful in the biomass and quality properties of medicinal and aromatic plants (Mitchell and Abernethy, 1993; Mitchell et al., 1995; Pank, 1992; Singh et al., 2011; Zheljzakov et al., 2006, 2010; Zumelzu et al., 1999).

Although weeds are reported as a problem by many producers in growing medicinal and aromatic plants (Lubbe and Verpoorte, 2011), the use of herbicides is not preferred in Europe and Turkey (Kristiansen et al., 2001). The most effective method in these areas, which can be called partly organic production, is the manual removal of weeds (Carrubba and Militello, 2013; Kristiansen et al., 2003); however, it is a time- and labor-consuming method and the need for 200-600 hours of human resources per hectare has been estimated (shifted to 1992). In some weed species, such as sage, the control of weeds before planting

comes to the fore (Karamanos, 2000). For crops such as true aloe, farmers in Mexico allow goats and lambs to graze in weeds because these animals do not prefer to feed on aloe (Cristiano et al., 2016). Mulching or flame methods can be integrated into existing applications (Junior et al., 2022).

### 3. CONCLUSION

Medicinal and aromatic plants are used for the human, animal, and even plant health. When the health effects of synthetic chemicals are considered, it is foreseen that the importance and agriculture of medicinal and aromatic plants, which are the active ingredient of many of the drugs used today, will increase. However, medicinal and aromatic plant farming weeds are an important biotic factor limiting production. These weeds vary according to the crop grown. They are single-year broadleaf (*Amaranthus retroflexus*, *Chenopodium album*, *Polygonum aviculare*, *Sinapis arvensis*), single-year grass (*Alopecurus myosuroides*, *Avena sterilis*, *Bromus tectorum*, *Lolium rigidum*, *Poa annua*), perennial wide leafy (*Cirsium arvense*, *Convolvulus arvensis*, *Plantago lanceolata*, *Prosopis farcta*, *Rumex crispus*), perennial grass (*Cynodon dactylon*, *Cyperus rotundus*, *Phragmites australis*, *Sorghum halepense*) and parasitic weed (*Cuscuta* spp., *Orobanche* spp.) species. These weeds compete with the plant grown for light, water, mineral matter, and place, causing the yield and quality of the product to decrease. To prevent these economic losses, weed control is a basic, technical, and economic necessity, accounting for almost 35-40% of the total production cost. Manual collection, hoeing, mowing weeds with

human resources, pre-planting/pre-planting tillage with a tractor and mixing weeds with soil, mowing germinated weeds, or tillage between rows are commonly used physical/mechanical methods of management. However, manual sorting from mechanical methods is impractical and economical, as it is laborious, takes a long time, and requires excessive labor.

Tractor tillage and mowing methods are also not sustainable due to excessive tillage, increased carbon emissions, and the high fuel cost. Using herbicides for the chemical control of weeds in medicinal and aromatic plants is seldom preferred. There is no licensed herbicide in Turkey in this regard. Although biological control and the use of bioherbicides are the critical control methods that need to be investigated, it is seen that this issue is not emphasized sufficiently. Competitive variety selection, alternation, allelopathy, shroud plant, mulching, solarization, thermal, robotic, precise, and intelligent weed control methods from alternative methods need to be integrated into existing applications. As a result, it is seen that human power and mechanical techniques come to the fore in the control of weeds, which are the problem of weed species and their management in the fields of medicinal and aromatic plant production and the problem of insufficient studies both in the world and in our country. Therefore, we need to identify problematic weeds in some essential medicinal and aromatics cultivated in our country to determine the management methods applied and integrate the appropriate ones with modern alternative tactics.



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## CHAPTER 7

### PHYTOREMEDIATION USING MEDICINAL PLANTS

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## **INTRODUCTION**

Heavy metals are among the environmental pollutants found in all industrial sectors (Lasat, 2002). The density of heavy metals is more than 5 grams per cubic centimeter. Heavy metals include lead (Pb), nickel (Ni), molybdenum (Mo), cadmium (Cd), copper (Cu), manganese (Mn), silver (Ag), iron (Fe), arsenic (As), Chromium (Cr), mercury (Hg), magnesium (Mg), zinc (Zn), selenium (Se) and cobalt (Co). Due to the fact that heavy metals cause poisoning and accumulate in food chains, so it is necessary to pay global attention in terms of environment and health (Adriano, 2001). Heavy metals are more durable in the soil than other parts, so soil contamination with heavy metals can cause many problems in the long run (Lasat, 2002). One of the important issues with heavy metal contamination is that heavy metals are non-degradable. This has led to heavy metals being considered as very dangerous pollutants in the environment (Kabata-Pendias, 2001). Although heavy metals are needed in small quantities for plants, but if they accumulate in larger amounts in plants, they can have negative effects on plant growth and cause various anomalies (Kabata-Pendias, 2001). Many enzymes, such as alcohol dehydrogenase, carbonic anhydrase, superoxide dismutase, carboxy peptidase, aldolase, proteinase and RNA polymerase require zinc to be synthesized. Zinc is necessary for the synthesis of growth hormone, ie auxin, protein metabolism, starch formation, chlorophyll production, membrane stability, DNA and RNA structure stability, carbohydrate metabolism, etc (Rudani et al., 2018). Copper is important for the natural growth and development of plants. Copper acts as a cofactor in



many enzymes, such as copper / zinc superoxide dismutase, cytochrome c oxidase, amino oxidase, plastocyanin, and polyphenol oxidase (Yruela, 2005). Copper plays an important role in photosynthesis and respiration, pollen constitution and grain production, rooting, water and nutrient uptake (Havlin et al., 2015).

If the concentration of metals in the soil is higher, toxic and dangerous effects will occur and prevent the growth of the plant. Heavy metals are obtained naturally through weathering of rocks and volcanoes, and humans also increase metals in the environment and pollution through the extraction of metals from mines, the smelting of metals and the use of pesticides and fertilizers containing phosphorus, as well as the burning of fossil fuels (Spiegel, 2002). A summary of the effects on plants by the heavy metals cadmium, chromium, copper, mercury, nickel, lead, and zinc is given in Table 1.

In recent years, due to human activities, the amount of heavy metals in the environment has increased significantly. Researchers have found that heavy metals can exhibit toxic effects and affect human and animal health. Heavy metals have been shown to cause cancer and reduce fertility. Therefore, it is necessary to use strategies to reduce heavy metal contamination. One of these strategies is the use of phytoremediation technology (Nriagu and Pacyna, 1988; Snow, 1992; Alloway and Ayres, 1993; Pacyna, 1996).

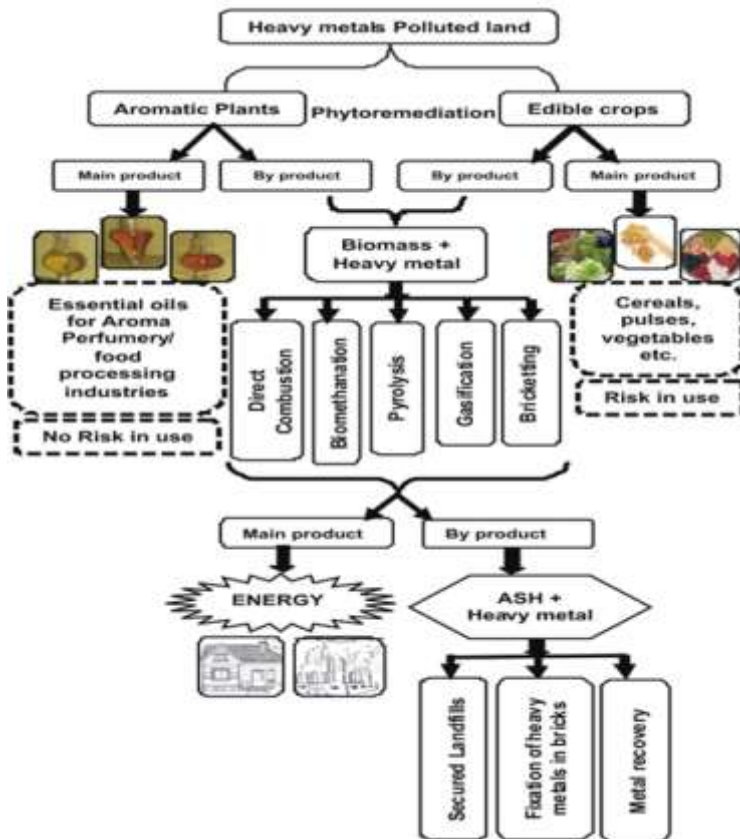
**Table 1.** The main effects of heavy metals on plants

Metal	Effects
Cadmium (Cd)	Prevent seed germination and root growth, prevent respiration, photosynthesis, reduce photosynthetic pigment synthesis and increase oxidative damage (Zhao et al., 2021)
Chromium (Cr)	Seed germination is delayed, root growth is reduced, biomass is reduced, membranes are damaged, leaves become chlorinated and necrotic, seed yield is reduced and plant death occurs (Amin et al., 2013)
Copper (Cu)	Inhibits photosynthesis, plant growth and reproductive process; decreases thylakoid surface area (Jorge et al., 2005)
Mercury (Hg)	Photosynthetic light and dark reactions are disrupted, plasma and vacuolar membranes are damaged, water transfer is disrupted, antioxidant enzyme activity is increased, accumulates phenol and proline (Patra and Sharma, 2000; Jorge et al., 2005)
Nickel (Ni)	Reduces seed germination, dry mass accumulation, protein production, chlorophyll production and reduces leaf area; increases free amino acids; Causes oxidative stress; affects the absorption of minerals (Jorge et al., 2005; Bhalerao et al., 2015)
Lead (Pb)	Reduces chlorophyll production and plant growth; increases superoxide dismutase; Prevent adenosine triphosphate production, lipid peroxidation and damage to deoxyribonucleic acid, inhibit seed germination, root elongation (Jorge et al., 2005; Kumar et al., 2015)
Zinc (Zn)	Reduces Ni toxicity and seed germination; increases plant growth and ATP/chlorophyll ratio (Jorge et al., 2005)

## 1. PHYTOREMEDIATION POTENTIAL IN SOME MEDICINAL PLANTS

Phytoremediation technology is important for refining and removing heavy metals from contaminated soils, as it is cheap, simple, generally accepted, and preserves soil properties. The hyperaccumulators are mostly wild plants that absorb a lot of metals but produce little biomass and have no economic value from an agricultural point of view. Therefore, these plants can not be used to clean the environment.

To treat soils contaminated with heavy metals, you can use edible crops such as corn, barley, which have a lot of biomass, tree and shrub species, and medicinal and aromatic plants. Medicinal plants may not be accumulators and may not have high phytoremediation potential. But for phytoremediation, medicinal plants can be used more (Figure 1).



**Figure 1.** The benefits of aromatic plants for phytoremediation (Gupta et al., 2013)

Phytoremediation is an efficient, low-cost and environmentally friendly way to remove heavy metals from contaminated sites. Plants

can treat pollutants through methods such as adsorption, transport and transfer of metals, mineralization, over-accumulation or conversion.

As edible plants are consumed by humans or animals, heavy metals enter the food chain through these plants. Therefore, it is not advisable to use these plants for phytoremediation because heavy metal pollution is not eliminated (Gupta et al., 2013).

Aromatic herbs are used in various industries such as food industry as a seasoning for food and spices and for the production of oil, in the pharmaceutical industry to fight diseases, in the cosmetic industry as a perfume and in agricultural industries to make natural pesticides and against pathogens. These plants are not edible and are not used directly by humans and animals. Aromatic plants that contain essential oils do not pose a threat to the accumulation of heavy metals. If phytoremediation is done by aromatic plants, the metals will not enter the food chain and the contaminants will be removed. In nature, these plants are not used orally because they contain essential oils (Gupta et al., 2013).

Phytoremediation is one of the most compatible and inexpensive methods that can be used to remove toxic soil contaminants such as lead (Pb). The choice of medicinal and aromatic plants in phytoremediation is important. Below are some of the medicinal plants used for phytoremediation of soils contaminated with heavy metals.

According to studies, lead metal reduce the biomass of three plants, blue mint (*Mentha aquatica* L.), eryngo (*Eryngium caucasicum*

Trautv.) and *froriepia* (*Froriepia subpinnata* Ledeb.). Blue mint root absorbs more lead than the shoot. In blue mint, the bioconcentration factor is higher than one and the transfer factor is less than one, so it can be used for plant stabilization of lead. In *froriepia*, the bioaccumulation factor and transfer factor is higher than one, so it could be used for plant extraction of lead. *Eryngo* has the highest lead uptake in the aerial parts. According to the results, all three plants in lead-contaminated soil have phytoremediation potential and *eryngo* show higher ability to plant lead extraction due to higher shoot dry weight (Hassanpour et al., 2020).

Cadmium is a non-essential divalent heavy metal cation. The toxic level of Cd may be caused by natural soil characteristics or by human activity. Cd has been shown to cause many morphological, physiological, biochemical and structural changes in growing plants. Meanwhile, some plant species can grow in Cd-contaminated soil and are able to uptake and accumulate this element in their tissues (Ghaderin and Hajjani, 2010)

The effect of cadmium on basil (*Ocimum basilicum*) show that when the amount of cadmium increases, the length of the roots decreases and the length of the stem increases. Cadmium also reduces the dry and wet weight of the plant, reduces the relative water content, leaf area and leaf area. Basil stores most of the metal in its roots. Although basil is able to accumulate high amounts of cadmium, it is less effective in clearing soil cadmium due to low biomass production (Nazarian et al., 2016).

The combination of nickel and lead has a negative effect on the dry weight of plants. According to the results, the roots of indian mustard and fenugreek contained more lead and nickel. Ethylenediaminetetraacetic acid (EDTA) cause an increase in lead in the roots and shoots of plants. In fenugreek, Bioaccumulation factor in lead and nickel composition is more than one and bioaccumulation of lead and nickel in fenugreek is higher than Indian mustard. The lead and nickel transfer factor in Indian fenugreek and mustard was less than one. According to the results, two plants accumulate lead and nickel in their roots and show low transfer to their shoots and can be used in phytoremediation (Kaur, 2018).

Studies on quinoa (*Chenopodium quinoa* Willd) show that with increasing levels of cadmium and lead, plant height, root and stem dry weight decrease. Quinoa roots and seeds have maximum and minimum cadmium, respectively. With the increase of cadmium and lead, the activity of antioxidant enzymes increase and the stability of the membrane is reduced. In fact, quinoa has a good capacity for phytoremediation of cadmium and lead (Amjad et al., 2021).

Plants used as food by humans and animals are not suitable for phytoremediation of soils contaminated with heavy metals. Because these plants accumulate heavy metals in their tissues and enter the food chain through consumption and can have negative effects on human and animal health. Medicinal plants can also be used in phytoremediation of heavy metals. Medicinal plants are mostly processed to extract

essential oils, and therefore heavy metals absorbed by medicinal plants do not enter the essential oil (Angelova et al., 2015).

According to the results, when *Hypericum perforatum* seedlings are exposed to chromium for 7 days, the levels of protopsudohypericin, hypericin and pseudohypericin increase. Chromium at a concentration of 0.1 mM for 7 days cause symptoms of toxicity in seedlings (Tirillini et al., 2006).

The results show that the highest amounts of heavy metals nickel, copper and manganese are present in *Artemisia vulgaris*, zinc in *Stevia rebaudiana*, iron in *Withania somnifera* and chromium in *Galium aparine*. The results show that due to the accumulation of heavy metals in medicinal plants, if medicinal plants are used by humans or medicinal products are prepared from them, they should be collected from a habitat without contamination (Khan et al., 2008).

The results of studies on *Peganum harmala* show that with increasing the amount of heavy metals cadmium, lead, zinc and chromium, the percentage of seed germination decreases. The toxicity of metals is in the form of cadmium > lead > chromium > zinc. Cadmium shows the highest toxicity, and zinc shows the lowest toxicity. In this study, radicle growth was further affected by heavy metal toxicity. It seems that the seeds of the plant show good resistance to the metal zinc (Nedjimi, 2019).

Lavender is a plant that accumulates heavy metals. Lavender is a hyperaccumulator of lead and is an accumulator of cadmium and zinc.

For this reason, this plant can be used for phytoremediation of soils contaminated with heavy metals. The results show that a small amount of heavy metals remain in the roots of lavender and most of the heavy metals are transferred to the shoots of the plant (Angelova et al., 2015).

Studies show that the concentration of aluminum in *Rosmarinus officinalis*, cobalt concentration in *Alpinia officinarum*, nickel concentration in *Mentha piperita*, lead concentration in *Malva sylvestris*, chromium concentration in *Cuminum cyminum* and cadmium concentration in *Thymus vulgaris* are higher (Esetlili et al., 2014).

The medicinal plants basil, peppermint and dill are able to grow in soils contaminated with the heavy metals cadmium, lead and copper. Also, heavy metals accumulated in plants are not transferred to essential oils and do not change the composition of essential oils (Zheljazkov et al., 2006).

In a study, three medicinal plants, *Thymus vulgaris*, *Thymus serpyllum* and *Salvia officinalis*, were compared in terms of metal content. *Thymus vulgaris* L. had the highest concentrations of lead, nickel and copper. *Salvia officinallis* also had the highest levels of manganese, zinc and iron (Abu-Darwish, 2009).

According to the results, with the increase of nickel, the amount of sugar and leaf protein in coriander plant decrease and the amount of phenols, flavonoids and flavonols increase (Goharriz et al., 2015).



Accumulation of heavy metals in different parts of medicinal plants is different. Chamomile (*Matricaria recutita*), sage (*Salvia officinalis*) and thyme (*Thymus vulgaris*), heavy metals such as cadmium, lead and nickel accumulate in the roots more than the shoots. Although heavy metals accumulate in medicinal plants, the quality of essential oils of medicinal plants is not affected and the essential oils are not contaminated with heavy metals. Therefore, in areas contaminated with heavy metals, the cultivation of medicinal plants can be considered (Lydakias-Simantiris et al., 2016).

The heavy metals in sage (*Salvia officinalis*) reduce the dry weight of roots and shoots. Sage plants absorb more zinc than copper, lead and cadmium. Due to heavy metals, the yield of essential oil increases and the components of essential oil decrease (Stancheva et al., 2009).

The results show that *Calendula officinalis* is resistant to copper. As the concentration of copper in the soil increases, so does the concentration of copper in the plant. Copper accumulation in shoots is higher than roots. However, at the highest copper concentration, copper transport to the shoot is reduced. Copper at higher concentrations reduces root and shoot weight, leaf area and root length. In fact, *Calendula officinalis* shows good phytoremediation performance and can be considered a hyperaccumulator plant (Goswami et al., 2016).

*Melissa officinalis* has a good capacity for phytoextraction and phytostabilization. According to the test results, the heavy metals

cadmium, arsenic and lead in the stem and seeds of this plant have the highest and lowest values, respectively (Aransiola et al., 2021).

Coriander (*Coriandrum sativum* L.) has a high resistance to copper metal, so this plant has the ability to phytoremediate copper-contaminated soils. The results show that when the copper concentration increases to a desirable level (300 ppm), coriander growth also improves (Özyazici and Bektas, 2021).

Two plants, *Bidens pilosa* and *Plantago lanceolata* extracts and concentrates higher concentrations of copper metal from the soil. Both plants are efficient in phytoremediation of copper contaminated soils (Andreazza et al., 2015).

Canola plant (*Brassica napus* L.) in the presence of zinc metal can increase its biomass. Due to the fact that canola can grow in high concentrations of zinc, so it is resistant to zinc contamination and is considered a hyperaccumulator plant (Belouchrani et al., 2016).

Researchers have found that vehicle traffic contaminates plants with heavy metals. The results show that *Achillea wilhelmsii* plant has more heavy metals in the roots. *Cardaria draba* stores metals in its aboveground part. *A. wilhelmsii* is able to transport copper and zinc metals to the aboveground part, and *C. draba* is able to transport cadmium, lead and copper metals (Hosseini et al., 2020).

Milk thistle (*Silybum marianum*) has shown a high ability to transfer cadmium to the shoot. Cadmium and diesel oil reduce fresh and

dry weight of shoots, fresh and dry weight of roots. Root biomass is more sensitive to cadmium than shoot biomass. Diesel oil also causes cadmium to accumulate more in the plant (Hammami et al., 2018). Protein content decreases significantly at 900  $\mu\text{M}$  cadmium concentration, while the activity of phenylalanine ammonia lyase, glutathione reductase and ascorbate peroxidases increases at this level. The results show that this plant has different mechanisms to reduce cadmium toxicity. The study shows that the concentration of cadmium is higher in the leaves and there is a small amount in the roots. According to the results, this plant has the phytoremediation ability (Poortabrizi et al., 2018).

*Solanom nigrum* has a very high ability to accumulate lead and zinc. *Thlaspi arvense* absorb cadmium better than other species. *Eruca sativa* and *Brassica napus*, because of their good growth, high biomass production and higher accumulation of lead, zinc and cadmium, can be said that these species are suitable for phytoremediation (Nasouhi and Ghaderian, 2015).

According to the results, in marjoram (*Origanum vulgare*), lead metal accumulates in the aerial part of the plant. Due to heavy metal contamination, the ratio of shoot to root dry weight increase in marjoram, and this makes the plant suitable for phytoremediation. The results show that the heavy metals cadmium and lead accumulate in the roots of basil (*Ocimum basilicum*) and due to the lack of accumulation of these metals in the aerial parts, basil can be used by humans without any danger. Due to heavy metal contamination, the leaf blades of the

plants became thicker. Heavy metals increase the amount of phenols and flavonoids in basil and increase glutathione, ascorbate and phenols in marjoram (Stancheva et al., 2014).

The results show that when the soil cadmium concentration increases, the roots and shoots of *Echinacea purpurea* absorb more cadmium but less cadmium is transferred from the roots to the shoots. Increasing cadmium concentration decreases leaf number, plant length, fresh and dry weight of shoots, but with 18% and 22% increase in fresh and dry weight of roots, high cadmium resistance is observed. Also, increasing cadmium in soil increases ion leakage by 11% and reduces antioxidant capacity by 43%. In general, *Echinacea* is able to treat cadmium-contaminated soil up to 25 mg/kg through high resistance to cadmium (Heidari et al., 2018).

Artichoke (*Cynara scolymus* L.) is resistant to lead and can accumulate lead and transfer it to their shoots. The results show that with increasing concentrations of lead, root and shoot biomass, chlorophyll content and artichoke leaf area index decrease significantly. Increasing the concentration of lead increases the uptake and accumulation of lead. Therefore, artichoke can be used to eliminate moderate lead pollution (Karimi et al., 2013).

According to the results of studies, with increasing lead uptake, biomass of roots and shoots in German chamomile (*Matricaria chamomilla*) decreases. As the concentration of lead increases to 180  $\mu\text{mol}$ , the concentration of this metal in the roots and shoots of German

chamomile increases. In fact, with the accumulation of lead in the roots, especially in the early stages of growth, this species has the ability to prevent the transfer of this metal to the aerial parts and the occurrence of toxicity in the plant (Saderi et al., 2011).

The results of study show that marshmallow (*Althea officinalis*) is a suitable plant for lead extraction and can be used to purify lead contaminated soils (Kolah Kaj and Mohammadi Roozbehani, 2017).

## **2. THE EFFECT OF MYCORRHIZA FUNGI AND OTHER MICROORGANISMS ON PHYTOREMEDIATION**

In recent years, various industries have increased soil and water pollution with heavy metals, and if the situation continues like this, the import of agricultural products contaminated with heavy metals can cause serious harm to humans and animals. For this reason, it is necessary to pay attention to soil purification. One of these low-cost and environmentally friendly methods is phytoremediation by microorganisms, especially mycorrhizal fungi. Studies show that mycorrhizal fungi can increase plant resistance to heavy metals (Cabral et al., 2015).

Microbial inoculation improves plants growth and increases their tolerance to environmental stresses and induces phytoremediation of heavy metals contaminated soils. Numerous studies have shown that symbiosis with mycorrhiza fungi increases phytoremediation power in soils contaminated with heavy metals. Studies show that glomalin produced by the mycorrhiza fungus is a glycoprotein that can stabilize

toxic elements such as copper, lead, and cadmium, reducing access to toxic elements and reducing toxicity to other soil microorganisms. Plants also grow easily in these places (Gonzalez et al., 2004).

There are different mechanisms in relation to plant symbiosis with mycorrhiza fungi and accumulation of heavy metals. These include the accumulation of heavy metal within the apoplast, ionic contact with compounds in the cell wall, accumulation of phytochlates in the cytoplasm and esterification, and finally through the metabolic processes of ion transport in vacuoles. Arbuscular mycorrhizal fungi in association with plant roots may affect plant growth, nutrition and tolerance to heavy metals in polluted soils. Therefore, this association can play an important role in phytoremediation.

Mycorrhizae in cadmium-containing medium has a positive effect on plant growth and protein content. Higher concentrations of cadmium prevent mycorrhiza activity. Mycorrhizal symbiosis in fenugreek reduces the transfer factor. Mycorrhiza also activates the antioxidant system in the plant, thereby reducing the harmful effects of cadmium. In general, fenugreek is able to withstand cadmium due to mycorrhizal symbiosis and keep the cadmium concentration in its aerial parts low (Abdelhameed and Metwally, 2019).

Mycorrhizal fungi in symbiosis with licorice plants cause the accumulation of lead and cadmium in the roots of plants. In fact, mycorrhiza absorbs heavy metals and stabilizes them at the root. According to the results, licorice is not a super-accumulating plant.

Also, with increasing the concentration of cadmium and lead, the activity of mycorrhiza decreases and the coexistence of mycorrhiza with different plant ecotypes is different (Tabrizi et al., 2020).

In the case of vetiver, with increasing lead content, percentage of root colonization, root dry weight and stem dry weight decrease, but due to mycorrhizal symbiosis, root dry weight and stem dry weight increase. Due to mycorrhizal symbiosis, plant uptake of lead increases. Due to the fact that in vetiver, the amount of lead accumulation in the roots is higher than the stem, it can be said that this plant is more effective for phytostabilization (Bahraminia et al., 2015). Studies by Wong et al. (2007) on vetiver show that the use of arbuscular mycorrhizal fungi increases the uptake of zinc and lead at low concentrations, but at high concentrations, the uptake of metals decrease. Toxicity due to high lead and zinc by mycorrhizal fungi is prevent.

Studies show that the arbuscular mycorrhizal fungus *Glomus intraradices* is able to stabilize the metals zinc and cadmium and transfer them to the roots of the carrot plant. Zinc metal is transported like phosphorus and cadmium is absorbed and transported through zinc and manganese transport systems. According to the results, mycorrhiza fungi increases phytoremediation of heavy metals (Giasson et al., 2005).

Arbuscular mycorrhizal fungi has a good coexistence with Rhizobium bacteria and this coexistence increases *Sesbania rostrata*

biomass and uranium accumulation in the plant. In this symbiosis, maximum uranium is removed and extracted. In fact, fungal-bacterial coexistence increases the efficiency of uranium phytoremediation (Ren et al., 2008). Mycorrhiza fungi has a positive effect on plant growth, increases the amount of organic chelates and develops resistance to arsenic metal (He and Lilleskov, 2014).

When mycorrhiza is not used, cadmium can cause toxicity in celery. Mycorrhiza (*Glomus macrocarpum*) promotes better plant growth and prevents cadmium toxicity. Cadmium reduces the dry weight of plants. With the application of mycorrhizae, the accumulation of cadmium in the shoot is reduced. The rate of cadmium accumulation in the roots of mycorrhizal plants is higher than non-mycorrhizal plants. Mycorrhizae increase phosphorus uptake and chlorophyll concentration of plants (Kapoor and Bhatnagar, 2007).

Based on the results, mycorrhizal fungus (*G. intraradices*, *G. mosseae*, *G. fasciculatum*) and *Pseudomonas* increase a number of parameters such as proline and root and shoot biomass in *Hyoscyamus* (*Hyoscyamus niger*). Lead extraction by shoots and lead stabilization in roots increase by fungal and bacterial application. According to the results, to increase the phytoremediation efficiency of lead by *Hyoscyamus*, mycorrhizal fungus and *Pseudomonas* bacteria can be used (Karimi et al., 2013).

The results show the phytoremediation ability of thyme (*Thymus daenensis*) to absorb heavy metals from contaminated soils. Thyme



roots and shoots accumulate more lead and cadmium, respectively. Comparing the concentration of lead in the roots and shoots of thyme, it can be seen that this plant has more of the root-stabilizing properties of lead metal and transfers smaller amounts of them to the shoots. The highest concentration of cadmium in plant shoots is obtained in cadmium-polluted soil and inoculated with *G. mosseae* and in roots inoculated with *G. fassiculatum*. Considering that mycorrhizal symbiosis increases the uptake of lead and cadmium, especially in the case of cadmium, the concentration of which in the shoot is higher than the root; the use of mycorrhizal fungi is recommended to increase the efficiency of soil phytoremediation (Mohkami et al., 2018).

According to experiments, with increasing the amount of lead and cadmium in the soil, the yield of marigold (*Calendula officinalis*) decreases and the negative effects of cadmium are greater than lead. Mycorrhiza causes more heavy metals to accumulate in the roots and shoots of marigold (Tabrizi et al., 2015).

### **3. THE EFFECT OF CHEMICAL FERTILIZERS ON PHYTOREMEDIATION**

Consumption of chemical fertilizers can increase plant biomass and metal uptake by the plant. In this regard, the amount and sources of potassium fertilizers are of particular importance. Potassium is the most important cation in plant physiology and metabolism not only in terms of the amount present in plant tissues but also in terms of physiological and chemical functions and activation of plant enzymes.

Young leaves in the lavender plant absorb more lead and cadmium. The transfer factor is higher than one because the concentration of metals in the stems is higher than in the roots. As the amount of black and green tea residues in the soil increases, so does the amount of lead and cadmium in the roots (Ziarati et al., 2014).

Based on findings, *Pteris vittata* L. biomass decreases with increasing arsenic. In arsenic-contaminated soils, phosphorus fertilizers such as calcium magnesium phosphate, calcium dihydrogen phosphate, and diammonium phosphate have higher biomass. Arsenic phytoremediation using calcium dihydrogen phosphate fertilizer is maximal. That is, with phosphorus fertilization, the efficiency of plant phytoremediation increases (Liao et al., 2008).

It was found that phosphorus fertilizer has a positive effect on plant biomass and arsenic accumulation in *Pityrogramma calomelanos*. In fact, phosphorus fertilizer is an important factor for plant extraction of arsenic (Jankong et al., 2007).

Urea and chicken manure have a positive and stimulating effect on the growth of shoots of *Solanum nigrum*. According to the experimental results, with the addition of soil cadmium, more cadmium is stored by the roots and aerial parts of the plant. Cadmium storage in plant shoots is maximal. Cadmium also has a negative and inhibitory effect on plant biomass. When urea fertilizer is tested, it does not affect the amount of metal in the plant. However, the use of chicken manure

is able to reduce the concentration of metal in the plant, which means that it reduces the cadmium extracted by the plant (Wei et al., 2010).

Phosphorus fertilizer has a positive effect on the dry weight of *Ricinus communis*. Phosphorus is able to increase the amount of copper in the shoot of the plant. Too much phosphorus reduces the concentration of iron in the plant, in other words, iron absorption is inhibited by phosphorus consumption. An increase in chlorophyll and carotenoids is also observed due to phosphorus consumption. Since the amount of copper in this plant is more than other accumulators, it can be said that it has a good capacity for phytoremediation. In fact, phosphorus fertilizer has a positive effect on plant growth under copper stress conditions. The results show that phosphorus fertilizer increases the phytoremediation efficiency of copper contaminated soil due to its positive effect on plant biomass and copper accumulation in the plant (Zhou et al., 2020).

According to the results, in the absence of fertilizer application, heavy metals lead, cadmium and zinc are accumulate in smaller amounts in the leaves and seeds of the milk thistle. Due to the application of ammonium nitrate fertilizer, the amount of lead in the seeds is maximized and potassium chloride also causes the amount of lead in the leaves to be maximized. Simple superphosphate fertilizer also causes less cadmium to be stored in the leaves of the plant, and in the case of a combination of the three fertilizers, less cadmium accumulates in the seeds. Ammonium nitrate maximizes leaf and seed cadmium content. In the case of zinc metal, more zinc is obtained in the

plant through the use of ammonium nitrate. Potassium chloride causes less zinc to be stored in the leaves, and the combined use of three fertilizers also reduce the amount of zinc accumulation in the seeds (Razanov et al., 2020).

According to the results of the experiments, in the soil contaminated with cadmium and lead, the concentration of cadmium and lead in the shoots and roots of the lavender plant is maximized. Application of potassium sulfate, potassium chloride and potassium nitrate fertilizers can increase phytoremediation efficiency. The effect of potassium chloride fertilizer is greater in phytoremediation. According to the results, lavender has the ability to absorb heavy metals and also the accumulation of cadmium and lead in lavender root is higher than the shoot (Mohammadian et al., 2016).

#### **4. THE EFFECT OF BIOCHAR ON PHYTOREMEDIATION**

Biochar is a carbon-rich product that is prepared under heat treatment with low oxygen or no oxygen, and their use in agricultural lands can be beneficial both in terms of agriculture and the environment. Biochar can remain in the soil as a carbon reserve for hundreds to thousands of years. Research has shown that biochar has a major effect on reducing greenhouse gases and improving global warming by carbon sequestration. Biochar has the ability to combine with organic and inorganic materials. Biochar with organic ligands can reduce the mobility of these metals in the soil, create complexes with

heavy metals and reduce their transfer to plants. Biochar also has a positive effect on plant growth and biomass.

In the study of the effect of bamboo biochar on the phytoremediation of *Salix psammophila*, it was found that biochar is able to increase soil organic matter and potassium and reduce the amount of heavy metals in the soil. The accumulation of cadmium and zinc were higher in the leaves and the accumulation of copper and lead were higher in the roots. Bamboo biochar increased the amount of copper in the roots and decreased the amount of lead in the plant (Li et al., 2020).

Studies show that the biochar of tea waste causes the accumulation and transfer of cadmium in ramie seedlings. Tea biochar is able to increase plant growth and reduce oxidative stress and reduce cadmium toxicity. In fact, biochar increases the efficiency of phytoremediation (Gong et al., 2019).

Both biochar and bacteria reduce the amount of heavy metals alone, but biochar and bacteria combined reduce the amount of metals several times more. Biochar and microbes reduce the accumulation of metals and increase plant growth (Andrey et al., 2019).

The results show that cadmium has a toxic effect on the growth of lavender. Cadmium is absorbed by the plant and accumulated in the roots of the plant. When soil cadmium concentration increases, the amount of cadmium in leaves and stems increases and the amount of cadmium accumulation in leaves is higher than stems. With the use of

biochar, cadmium is slightly transferred to the stem and leaves, especially very little cadmium is transferred to the stem. Lavender absorbs less cadmium due to the use of biochar, thus avoiding cadmium toxicity. In fact, because biochar has a high surface area and high cation exchange capacity, it can absorb cadmium from the soil solution. Based on findings lavender is a hyperaccumulator plant (Hashemi et al., 2017).

Application of biochar and inoculation of AMF increase dried weight of shoots and roots of peppermint (*Mentha piperita* L.). Also application of biochar increases the percentage of essence in all AMF inoculated peppermints. Comparison of interaction effects of biochar and Cd show that application of biochar with Cd at 100 mg/Kg level, has the highest Cd accumulation in root (Rezaian, 2014).

## **5. THE EFFECT OF CHELATORS ON PHYTOREMEDIATION**

Harvesting and maximum extraction of contaminants from soil environment is the purpose of phytoremediation. Use of chelates, is one of the effective approaches for increasing the bioavailability of heavy metals. Chemical and natural chelators can increase the uptake of heavy metals by plants. Chelating agents have a strong affinity for different heavy metal cations and they are easily transported from the roots to the shoots in the form of a metal complex with chelates. Addition of chemical chelators such as citric acid and EDTA and natural chelators such as poultry manure extract, cow manure extract and sheep manure extract to the soil has increased the usability of metals and ultimately their absorption and storage by plants.

According to experiments, with increasing copper content, root and stem growth, leaf area and number of leaves in *Calendula officinalis* decrease. Copper accumulates more in the stems than in the roots. Due to the fact that the transfer factor and bio-concentration factor are more than one, the plant is considered a copper-resistant plant and is considered a suitable plant for plant extraction. Consumption of salicylic acid as a chelate can increase the growth of plants and increase their resistance to copper. In fact, salicylic acid has a positive effect on phytoremediation (Afrousheh et al., 2015).

Increased accumulation of lead in alfalfa leaves grown in hydroponic environment due to concomitant use of EDTA and indole acetic acid has been reported by Lopez et al. (2005).

Ethylenediaminetetraacetic acid (EDTA) acts as a chelating agent for heavy metals, increasing the copper content in plants and increasing biomass. Plant growth-promoting rhizobacteria (PGPR) alone have a positive effect on copper uptake and biomass growth. The combination of the mentioned bacteria and chelator, are also able to have the maximum effect on the absorption of copper and biomass. The Pumpkin plant accumulates more copper in its shoots, indicating that the squash is effective in plant extraction. Corn accumulates the maximum amount of copper in the roots, indicating that corn is a stabilizing plant. In general, Ethylenediaminetetraacetic acid and plant growth-promoting rhizobacteria (PGPR) are able to increase copper uptake and copper extraction by plants and increase phytoremediation efficiency (Abbaszadeh-Dahaji et al., 2019).

Consumption of tannic acid (TA) and ethylenediaminetetracetic acid (EDTA) increase the transfer factor and increase the concentrations of cadmium, nickel, copper and lead in *Althaea rosea* Cavan. Studies show that using EDTA reduces plant dry weight and this indicates that EDTA chelate can be toxic to plants and dangerous to the environment. Tannic acid also has no negative effect on the environment and is more effective than EDTA in terms of metal accumulation in plant shoots and increase phytoremediation efficiency (Cay et al., 2015).

Comparison of EDTA and citric acid shows that EDTA is more effective in phytoremediation than citric acid, but it is more likely that it does not decompose in the environment and remains in the environment. Citric acid also has no negative effect on the environment and increases the efficiency of phytoremediation (Shinta et al., 2021).

Kiarostami et al. (2019) examined the contamination resistance of *Lavandula* against lead through 3 completely randomized block design experiments. The treatments included: lead in 2 concentrations (0 and 100 mg) and DTPA (Diethylene triamine pentaacetic acid) in 2 concentrations (1 and 2 mM). The results showed that the stress of heavy metals reduces the morphological characteristics of the study and by adding DTPA chelate, the plant absorbed less metal.



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## **CHAPTER 8**

### **IMPORTANCE OF MEDICINAL AND AROMATIC PLANT INTEGRATION TO SUSTAINABLE SOIL MANAGEMENT**

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## **INTRODUCTION**

The implementation of “agroecological farming system” is based on a monolith natural resource management for food security, safety, and sovereignty (Altieri, 1999; Gallardo-López et al., 2018) and mainly focus on soil fertility. Under the framework of this concept, in “ecological (=organic=biological) soil management”, to understand the connections among the land, air, water, and plant is essential and need different strategies and unaccustomed measurements have been chosen unlike conventional agriculture. These complex relationships that exist in the nature is depend on the functioning of a whole integrated and inter-related system (Atkinson and Watson, 2000).

In practice, soil fertility improvement in organic agriculture relies on enhanced understanding of the effects of sustainable management on soil indicators. Simply, organic agriculture relies on the management of soil properties to enhance the chemical, biological, and physical properties of the soil, in order to optimise and maximise crop yield and quality. To understand the indicators, one of our innovations in management of organic soil fertility is to make “holistic approach success” visible by doing “intensive and long term agricultural research” activities.

Plant nutrition and soil fertility in organic farming include complementary methods of soil health that not only help to nourish and grow the plant, but also support the permanent fertility of the soil. Therefore, the success indicator of the applied method can be

determined at the end of a long chain reaction. The most important of these methods is the use of "medicinal and aromatic plants" in agricultural production. The integration of medicinal and aromatic plants in organic agriculture will be examined under three headings in this article. These topics are presented in the following items;

1. Intercropping,
2. Hedgerow management, and
3. Compost initial-organic material.

Following the planting of medicinal and aromatic plants' positive effects in intercropping systems on soil organisms, it comes to the fore in terms of causing the proliferation of microorganisms and especially the microorganisms responsible for the conversion of plant nutrients into available form (Adamovic et al., 2014; Golijan and Markovic, 2018). Due to plantation of medicinal and aromatic plant in dual cropping with main crop, soil microorganisms directly act to oxidise soil organic matter. These soil microorganisms interact bio-chemical cycles of carbon (C), nitrogen (N), phosphorus (P) and sulphur (S) (Balloni and Favilli, 1987; Mäder et al., 2000). In recent years, the importance of existing hedge management systems has been understood, especially in countries such as Turkey, where virgin and rural areas with existing polyculture agricultural land structure are rich in plant diversity. The nature of the natural vegetation, which is prone to hedgerow system without much effort, emerges as an important advantage. At this point, one step ahead is to seek to disseminate annual

and permanent plant species in all agricultural areas by working on planned plans as of today. What is emphasized in the section is the necessity of evaluating regenerative and carbon farming systems not only with agroecological and organic certificated systems, but also in a broader framework such as conventional. Furthermore, the aim to reach zero waste is cover the making compost measurement in also on-farm conditions as well as urban agroecological systems. In farm level, oxygen enriched co-composting of the plant artificial materials can be used in the compost pile to recycle the organic based garbages of the plant production in organic agriculture. One of the measurement to reach soil health in farms is to use mature compost in agroecological farming systems (Wezel and Silva, 2017) and maturity is defined reduced C:N ratio of the compost. Using medicinal and aromatic plant residues may support to obtain high quality and hygenic end product (Kir et al., 2021). Apart from the general advantages of intercropping, hedgerow implementation in the farms, and co-composting; in this section privatised the components of the organic soil management strategies are considered and the tools confined to be in the framework.

## **1. INTERCROPPING OF MEDICINAL AND AROMATIC PLANTS**

The growing of two or tree crops together or multiple cropping system which is called “intercropping”, has the possibility to reach developed yield and quality of crops (Vandermeer, 1992). Furthermore, the effective performance of the cultivated plants and conservation of soil, water and inputs such as recycled nutrients from the deeper parts

of the terrestrial areas can be results of the intercropping, if the cultivation design conducted properly (Wolfe, 2001; Glijan and Markovic, 2018). Significance of intercropping in organic soil management of medicinal and aromatic plants mainly sourcing from the availability of the organic nitrogen (Dikr, 2022). The crucial points of the medicinal and aromatic plants integration in cropping plans of the organic farms are related to (1) soil microbial symbiosis of the organism with this species (Badalingappanavar et al., 2018) and (2) ensure nutrient cycling (Innis, 1997). In general, organic management and rotation is found positive effects and enhance the microbiota community abundance and diversity in soils (Orr et al., 2011; Wang et al., 2012). In some cases, produced plant species effect mainly on soil microorganism composition (Grayston et al., 1998; Miethling et al., 2000). Cultivating medicinal and aromatic plants in multi-cropping systems may pave the way for recycling of the plant essential nutrients for “main crop” production. Adamovic et al. (2015) reported that the “mint” increased the total number of microorganisms, number of azotobacters, free nitrogen-fixing and cellulolytic microorganisms and reduced the number of ammonifiers in the soil samples. In soil samples of “marigold” production plots, it is found increase of the total number of microorganisms, ammonifiers and fungi. The number of ammonifiers were found higher in the “basil” and “dill” produced plots as it compared non-used control. It is concluded that to obtain a comprehensive knowledge, there is a need for a further research of monitoring of microbes involved in N and C cycle under different farming systems. The publication released in Idbella et al. (2022)

underlined the strong relationships among (a) soil chemical parameters, (b) soil bacterial and fungal composition and diversity, and (c) medicinal and aromatic plant litter chemical properties on the soil. Idbella et al. (2022) concluded to enhanced effects of *Pistacia lentiscus* L., *Juniperus phoenicea* L., *Myrtus communis* L., *Rosmarinus officinalis* L., *Olea europaea* L., and *Euphorbia dendroides* L. on soil microbiota. Moreover, intercropping could be an option to give rise to the soil parameters more relevant climate-smart agricultural results. For instance, Verma et al. (2014) reported that arecanut multiple cropping with medicinal and aromatic plants (*Aloe vera*, *Artemisia pallens*, *Piper longum*, and *Bacopa monnieri*) resulted in increasement of soil organic carbon level which means regenerate soil health. The microbial activity in soil is controlled by several factors as well as cultivated plant species, land carbon and plant essential mineral nutrients quantity, and soil microflora-microbiota interactions (Nannipieri et al., 2003; Barbosa et al., 2013). Sujatha et al. (2011), determined that the intercropping of medicinal and aromatic plants (*Aloe vera*, *A. pallens*, *P. longum* and *B. monnieri*) resulted in increasement of trial site-soil pH and the soil organic carbon (SOC) content, significantly. Likewise, another report also supported that intercropping with species of aromatic plants improved soil quality. It is found that a significant increasement in soil organic and available nitrogen contents, while a decrease ment reported in soil pH values (Chen et al., 2014). Zang et al. (2021) investigated intercropping influences to the soil microbiota. They used aromatic plants (*Ocimum basilicum*, *Satureja hortensis*, and *Ageratum houstonianum*) as intercrops that separately grew between rows of pear

trees, and no plants were grown as the control. root exudates from aromatic plants influenced the diversity, structure, composition and function of the soil microbial community, which regulated C and N nutrients during SOM mineralisation. Rodr guez-Lizana et al. (2020), studied C, N, P and K release from residues of *Brachypodium distachyon* and *Sinapis alba* used as cover crops in an Olive Grove in Spain. The 4-year trial average results indicated that after decomposition of the cover crops, C, N and P released to soil 40 to 58% of the C, N and P amounts in the residues after mowing. However, K was had the highest release to soil as 80–90%. Verma et al. (2013) reported a successful strategy using intercropping with potato and Geranium (*Pelargonium graveolens* L.). Significant variations were found in soil organic C, total organic N, microbial biomass content. The intercropping was established sustainability on productivity of the crops and soil health. On the other hand, medicinal and aromatic plant integration into sustainable main perennial production may support fruit yields. Ashraf et al. (2019) indicated that 1:4 ratios of pomegranate and aromatic plants (sweet basil and rosemary) promoted the yields under limited input application conditions. The intercropping plantation of arecanut trials conducted separately with different medicinal and aromatic plants of India (*Bacopa monnieri*, *Ocimum basilicum*, and *Artemisia pallens*). The productivity of the arecanut plots were found promisingly higher as it was compared the monoculture cropping system (Sujatha et al., 2011). In an olive orchard a case study result was reported (Anonymous, 2022a). In an olive orchard, oregano plants planted as an inter crop between the trees. In the first year, oregona

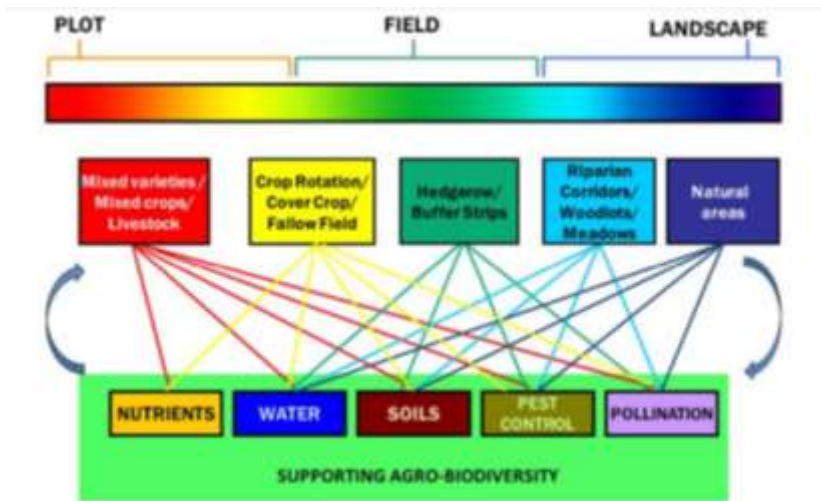
performed worse 3 tonnes) as the second year plant improvement was found well (5.5 tonnes) in the plot. Olive yield intercropped with oregano was recorded as 900 kg table olive and 4600 kg (500 kg processed extra virgin olive oil).

## **2. HEDGEROW MANAGEMENT**

The plant growth of edge of the field is an effective and extensive technique in agroecological systems. In this part, the best practices of medicinal and aromatic plants in the hedge management in organic farming. Whereas farming approach on “buffer strips” assure plant health, implementation of buffer strip in agrosystem has not cover only pest and disease management. Hedgerow management has also feature and maintain sustainable nutrient management. The harvest and pruning residues of medicinal and aromatic plants existing in the buffer strips may be a good strategy to use them in on-farm composting. In Figure 1, hedgerow implementation classified as one of the biological diversitation in the agricultural production ecosystems (Kremen et al., 2012; Thakur and Raj, 2006). For instance; In Asia, vetiver has traditionally been used as medicinal and aromatic plants in many countries in hedgerows of the fields. Farmers have indirect benefits from the “vetiver in hedgerow-production”. One of the reason they grow vetiver fort he leaves as by-products. Vetiver leaves grown as hedgerows for soil and water conservation have used as raw material for composting and mulching (Chomchalow, 2000).



Palada et al. (2004) reported another practise about vegetative performance and yield results for a hot pepper in hedgerow intercropping with *Morinda citrifolia* L. variety. It is concluded that hot pepper- *Morinda citrifolia* L. tree production in India was not showed any negative influence to hot pepper yield and quality.



**Figure 1.** The model of Sustainable farming systems

(Kremen et al., 2012).

### 3. INITIAL ORGANIC MATERIAL OF CO-COMPOST

Composting is an ecological based practice of organic garbage incorporation and decomposition which end up dark coloured, hygenic, stable and mature compost (Epstein, 1997; Filipović et al., 2013). Composting subsidise the circular economy because of returned valuable organic waste. Considering amount of small family farm apply “compost” to their production lands reusing and recycling the

biodegradable garbages in their farms, because green plant artificial materials and manure based compost effects on soil properties and crop yield-quality were intensively have worked in the research area and the positive influences have still reported in many publications (Watson et al., 2002). It is well documented in the literature that essential plant nutrients are available in crop residues and their recycling into a good quality-mature compost which has low C:N can be an important source as organic amendment in agricultural soils (Kir et al., 2021). The application of co-compost may lead to rapid mineralization and soil mineral N rise in the treated soil (Rahn et al. 1992). The fundamental plant macro (N, P, K, Ca, Mg) and micro (Fe, Cu, Zn, Mn, and B) nutrients are available in plant residues which can also to the soil depending on the crop species concerned (Wild, 1988; Sylvester-Bradley, 1993). Recently, EU Comission decided to support development of the recycled organic waste based soil conditioners and aiming an important step, objected to reach bigger organic recycled fertiliser market for organic agriculture under the framework of the Green Deal movements. EU Comission estimated that fertilisers made from organic waste could replace 30% of chemical fertilisers (Anonymous, 2022b).

In co-composting the biodegradable raw parts of the medicinal and aromatic plants are good sources for compost pile initial material (Saha and Basak, 2019). However, the residues have still been remained underutilised in many geographic areas of the earth (Filipović et al., 2013), due to in organic farming the medicinal and aromatic plants

collecting from the nature in a high amount (Willer et al., 2020). As a soil conditioner, compost develop not only chemical and physical properties of soil, but also lead up to enhancement in soil microbiota total content and diversity. Saha and Basak (2019), mentioned about additional advantage of the residues of spices. They drew an attention that the medicinal and aromatic plant fresh residues and by-products are cheap materials and co-compost made of the raw material of medicinal and aromatic plants, rock phosphate and manure had sufficient N, P, K levels of mature soil enhancer. Gezer et al. (2016) used a compost to produce mushroom made up of 43% wheat straw+ 43% poplar dust+ 10% wheat bran+ 4% gyps mixture was used as the control group, 20% to 40% of medicinal and aromatic residue was added to this formula in the experiment groups. Each medicinal and aromatic residue (*Laurus nobilis*), thyme (*Origanum onites*) and cumin (*Cuminum cyminum*) was applied as a treatment. The cumin added (20%, v/v) compost had higher (36%) biomass of the oyster mushroom as it is compared the non-used control (25%) compost material.

#### **4. CONCLUSIONS**

Improving information over agroecosystems is essential to develop future strategies compatible with the environment for the use of land, and achieve agricultural productivity and sustainability in soil health and medicinal and aromatic plant integration into the organic cropping actions. To a better and conserved soil fertility in production of organic agriculture, there is a need to identify and exploit “*main plant-accompanion medicinal and aromatic plant-soil fertility-soil life*”

relationships of intercropping addressing optimised soil nutrient cycling and use efficiency. By integrating medicinal and aromatic plants into the hedgerow system, the ground will be provided for practices that activate soil health. Accordingly, the biorecyclable garbage left on the farm and originating from the hedgerow will be an important resource for composting.

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## CHAPTER 9

### PLANT BREEDING BASED EVALUATION OF TURKISH TEA [*Camellia sinensis* L. (O.) Kuntze] GENETIC RESOURCES

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## INTRODUCTION

Tea (*Camellia sinensis* L.), is a evergreen perennial plant and belongs to the Theaceae family. This well-known plant was originated in China. Based on its global production areas, it can be stated that this plant has a wide adaptability, which has resulted in its production under varying agro-ecological conditions around the world.

Furthermore, tea (*Camellia sinensis*) is a significant economic crop (Xia et al., 2017). Its leaves are generally used to produce tea which is the world's most popular non-alcoholic beverage. Tea contains numerous characteristic compounds, such as tea polyphenols, caffeine and theanine, which not only determine the quality of tea but also confer significant health benefits on it (Wei et al., 2018).

In the thousands of years since the tea plant was discovered, recorded, and cultivated to produce tea in China, it has distributed to more than 50 countries worldwide in subtropical and tropical regions. Tea cultivars are highly heterogeneous due to their wide range of growing areas, long history of cultivation, allogamy and self-incompatibility of tea plants, resulting in high diversity in both their genetics and morphology (Chen et al., 2005; Kottawa-Arachchi et al., 2018). The availability of germplasms and genetic diversity within a gene pool, as a major element of crop genetic improvement, determine the outcome of breeding programs.

## **1. PRESENT STATUS REGARDING AGRICULTURAL PROBLEMS**

Global climate change is currently one of the most discussed topics in the world, and it is expected to persist. Tea growers are confronted with these anticipated changes in growing areas, and they anticipate major challenges (Nowogrodzki, 2019; Ranatunga, 2019).

Although climate change affects each region differently, it has a broad impact on tea yields by altering precipitation levels, increasing temperatures, shifting season timing, and stimulating insect pests (Nowogrodzki, 2019)

Rising temperatures and drier weather conditions are expected to reduce tea yield. Increases in temperature and the occurrences of severe weather events (sudden downpours, prolonged droughts) can potentially cause soil degradation and decrease soil fertility (Wijeratne, 2018). Furthermore, global climate change is expected to increase the prevalence of diseases, pests and weeds in these areas. (Lal, 2005). Besides that, in warmer tea-growing regions, insufficient exposure to low temperatures during the winter months may disrupt budding synchronization at the first flush (Tanaka, 2012). Budding synchronization is critical for maintaining the quality of first flush leaves. As a result, new tea cultivars suited to varying ecological conditions and cropping patterns are required (Wijeratne, 2018). Tea cultivation practices are labor intensive, and the availability of skilled labor in tea-growing regions is rapidly declining (Wijeratne, 2018). Most tea-growing countries are now mechanizing tea cultivation

practices such as land preparation, harvesting, and pruning. As a result, tea cultivars amenable to mechanization are in high demand, and crop improvement programs must account for this. The demand for a pure beverage among tea consumers is growing and will continue to grow; the use of agrochemicals is discouraged all over the world. Tea breeders are tasked with developing new tea cultivars with multiple pest and disease resistances while maintaining quality. Continuous and excessive application of inorganic fertilizer has a long-term impact on soil health (Wijeratne, 2018) and contributes to ground water pollution. (Tanaka, 2012). Hence, new tea cultivars with improved natural fertilizer usage efficiency needs to be researched.

Furthermore, due to health and environmental concerns, the movement toward organic tea farming has grown significantly in recent years (Hajra, 2018). As a result, in order to gain the benefits of organic agriculture, suitable tea cultivars for organic farming need to be developed.

## **2. ADAPTATION MEASURES FOR TEA CULTIVATION**

The following adaptation measures are recommended for tea cultivation:

- Planting drought and stress tolerant tea cultivars;
- Diversifying production, including converting low-yielding tea land from tea to other crops that can thrive in poor soil and in tea-growing areas;
- Intercropping tea with other tree crops, such as rubber, and/or other food crops. Shade trees protect the tea plants while also

providing shade. As the tree becomes older, it can be used as firewood to dry the tea. Food crops, on the other hand, supply the farmer with food and/or income. Intercropping with nitrogen-fixing crops like beans could also help improve soil. However, non-nitrogen-fixing crops such as cassava absorb nutrients from the soil and contribute to soil deterioration.

- Organic cultivation; and water conservation through efficient artificial irrigation and drainage systems, as well as water harvesting (Chang-Secretary and Brattlof, 2015).

### **3. BREEDING AIMS OF TEA (*Camellia sinensis*)**

Tea breeding strategies differ from country to country, based on local needs (Table 1). Therefore, the fundamental objective is to enhance quality and yield. In general, breeding studies in black tea-producing countries such as Kenya, India, and Sri Lanka are aimed toward the development of high-yielding, high-quality clones, whereas breeding efforts in tea-producing countries near the equator, such as China and Japan, are aimed toward the development of frost tolerance and cold tolerance, as these countries primarily produce green tea, where the quality of the finished product has little bearing on price. Modern tea cultivars have evolved over many years of dedicated work from both tea breeders and experienced planters at various stages of plant introduction, selection, hybridization, and chemical and physical breeding.

**Table 1.** Breeding objectives of tea

Objectives	Importance	Regions
Improving quality	Directly linked to the profitability	Black tea producing countries such as India, East-Africa, Sri Lanka, Bangladesh and Indonesia
Increasing yield	Horizontal increase of production by extension planting is limited	Worldwide
Drought tolerance	Reduce productivity and occur all tea-growing regions of the world	Worldwide where tea grown as rain-fed crop
Reduce winter dormancy	No leaf production during winter months and occurs in North-East India, Japan and China, etc	Tea plantation near the equator
Hail/frost resistance	Causes economic loss as young leaves during rainy season are mostly affected	Hilly region of the tea-producing countries
Water log tolerance	Reduce productivity during rainy season. Generally occurs in northeastern India	North-East India
Cold hardiness	Reduced productivity during winter due to snow. Generally occurs in China, Japan and Russia	Mainly India, Sri Lanka, Indonesia and Japan
Diseases resistance, such as blister blight, stem canker, etc.	Blister blight causes severe damage as only young leaves are infected. Generally occurs in Japan, Sri Lanka, South India and Darjeeling hills of North-East India	Mainly India, Sri Lanka, Indonesia and Japan
Pest resistance, such as red spider mite, tea mosquito bug, leaf-sucking pest, etc.	Most important biotic stress - causes severe damage to the leaves. Generally, in all tea regions in the world	Worldwide
Suitability to type of manufacturing	For matching the customer's demand as well as better recovery percentage in made tea	Black-tea-producing countries such as India, East-Africa, Sri Lanka, Bangladesh and Indonesia
Low input responsive Clone	Required for organic tea farming	Organic tea
Resistance to low soil pH		Worldwide

Mondal (2009, 2014)



#### **4. PROPAGATION OF TEA**

Tea can be propagated either vegetatively or by seed. Since two separate plants are normally required to generate the seeds, the progeny have a blend of the characteristics found in each parent, with each individual matching its parents to differing degrees. As a result, the descendants from seed are highly varied in appearance, yield, and other characteristics. According to Kingdon-Ward (1950), a major aspect in tea breeding programs was the selecting of potential plants from the current population as a resource of planting material production. (Green, 1970).

Tea is selfincompatible and outbreeding in most of the populations typically cultivated. The seed-bearing trees are largely obtained from random seedlings, and pollination in the orchard is unregulated. As a result, the seeds are heterozygous, and the seedling populations are exceedingly diverse.

#### **5. IMPORTANCE OF GENETIC RESOURCES**

Resources are definitely the key to crop genetic improvement in terms of tea plant germplasm collection and conservation (Xia et al., 2020). The quantity and distribution of genetic variants found in collections determines the success of plant breeding and conservation. Tea germplasms are rapidly becoming the most valuable basic material for tea breeding and biotechnology research, with enormous promise for the tea industry as a whole. According to partial information from the China Tea Science Society, extraordinary successes in international tea plant germplasm surveys, collecting, and conservation have

conserved more than 15,000 tea plant accessions. (<http://www.chinatss.cn/>). These invaluable germplasms have accelerated the progress of tea plant genomes, genetics, and breeding by a factor of (Taniguchi et al., 2014; Yang et al., 2016; Xia et al., 2019; Meegahakumbura et al., 2017; Koech et al., 2018; Koech et al., 2019). However, the majority of the present accessions collected and used are varieties, with only a few "wild" or close relatives incorporated. Another key issue that the sector is currently dealing with is the unequal use and protection of cultivars vs "wild" germplasms. As fast economic development and urbanization, the survival environment of "wild" tea resources has been continually degraded. Furthermore, due to the recent popularity of elite varieties, local germplasms (landraces) with distinct features are on the verge of extinction. Above all, tea plant germplasm surveying, collection, and conservation should be prioritized. Otherwise, no varied tea plant resources will remain, and only a few mono types will be available for future genetic advancement of tea. To hasten the preservation and usage of global tea plant resources, we recommend that a tea plant conservation alliance comprised of all tea-planting countries be formed (Xia et al., 2020).

## **6. DEVELOPMENT OF CORE COLLECTIONS**

If genetic resources are to be used effectively in crop improvement programs, they must be conserved and maintained in a systematic manner, followed by thorough characterization and evaluation.

Though a germplasm collection or genebank is intended to conserve the diversity of genetic resources for the benefit of plant breeders for germplasm enhancement through variety development, the lack of evaluation data prevents a germplasm collection from being used for crop improvement. Due to time and budgetary constraints, most tea germplasm accessions are not fully assessed for morphological, agronomical, and biochemical traits. Tea breeders face the challenge of selecting a broad and representative selection of accessions from a lengthy list of essentially anonymous accessions for cultivar development. One reason for tea breeding's reliance on such a limited genetic foundation could be this. As a result, building a core collection could be an appealing alternative for addressing the issues of tea genetic resource conservation and exploitation.

The number and size of germplasm collections of major crop plants continues to rise around the world. Originally, the collections were established to preserve the genetic diversity of cultivated species before much of it was lost forever as farmers replaced primitive stocks with contemporary, highly developed cultivars. Better access to and use of genetic resources in collections has been a major problem in recent years. Efforts to extend the use of gene-bank material in plant development have been hampered by the quantity and heterogeneity of collections.

## **7. HISTORY OF TEA IN TÜRKİYE**

Tea's history in Türkiye has begun the 1930s, the government decided to launch a poor farmer's agricultural program in the Northern Black Sea Region. Tea cultivation began predominantly in Türkiye after the 1940s, with the primary goal of meeting domestic demand. It was difficult at first to introduce the new crop, tea, into the specified area. People's general perception of the early attempts in tea cultivation was poor at the time. Despite this, Türkiye's domestic tea industry and tea commerce have grown into a profitable enterprise. In the meantime, Türkiye has established itself as a major player in the global tea industry (Klasra et al., 2007). Tea cultivation was originally started at Batum, as can be seen (Republic of Georgia). In the last quarter of the nineteenth century, Russians brought seedlings from China and developed plantations in Türkiye's Eastern Black Sea region. People said that after a successful tea growing in Georgia, the same could be done in Türkiye's north east. Following the successful introduction of tea by Russians in Batum, speculations arose that tea growing could be a viable choice for Turkish farmers. Tea cultivation was first attempted in Bursa in 1888, with plants imported from China and Japan (Tekeli, 1976). However, due to the unfavorable conditions for tea in Bursa, it was determined that tea plants need particularly specialized environmental conditions in order to produce a profit. Ali Riza Erten then began lengthy visits to Rize, Artvin, Ardahan (Türkiye), and Batum (Georgia) in the Eastern Black Sea region to investigate the viability of other appropriate locations for tea planting within Türkiye. (Kakuzu, 1944; Kacar, 1986a,b). He conducted a thorough

investigation and concluded that the ecosystem of Rize, Artvin, and Ardahan was quite similar to that of Batum. Following his investigation, he presented a report to the government stating that tea cultivation in Rize and its environs could be possible. (Hatipoğlu, 1934a,b; Arar, 1969). Tea planting was established in Türkiye in 1924, and the Tea Research Institute was founded. The first plant for processing green tea leaves was established in Rize in 1947. Tea is grown in the provinces of Rize, Ordu, Giresun, Trabzon, and Artvin. In order to provide better service in tandem with the sector's growth, the ÇAYKUR (General Directorate of Tea Enterprises) economic enterprise was founded in 1971 and was completely authorized as a state monopoly in the tea business. Private firms were allowed procurement, processing, and marketing rights in the tea sector once the monopoly was abolished in 1984 (Anonymous, 2017).

### **7.1. History of Tea Breeding in Türkiye**

ÇAYKUR was established in 10.10.1983 as a government institution. ÇAYKUR and its Atatürk Research Institute for Tea and Horticultural Cultures (Atatürk Çay ve Bahçe Kùltürleri Araştırma Enstitüsü Müdürlüğü) are responsible for tea cultivation and breeding in Türkiye.

### **7.2. Tea Breeding Activities in Türkiye**

ÇAYKUR, as a governmental institution, has the mission and responsibility for tea research and breeding in Türkiye. Different research aims were performed for farmers based on their needs. Two new tea research centers were established in the last decade. ÇAYMER

in Çayeli/Rize and the Tea Research and Application Center of the Recep Tayyip Erdogan University.

### **7.2.1. Tea Research Institutions in Türkiye**

#### **7.2.1.1. Rize Tea Research Institute**

This institute belongs to ÇAYKUR. The Institute's mission is to develop tea planting and production in Türkiye. Furthermore, it attempts to compete in all areas linked to improving and expanding the organization's competitive potential in both domestic and international marketplaces.

#### **7.2.1.2. ÇAYMER**

This center, ÇAYMER (Rize Çay Araştırma ve Uygulama Merkezi/Rize Tea Reserach and Application Center), has financial support from the EU and the Turkish government The overall goal of this initiative is to generate new research and value-added tea products in this region.

#### **7.2.1.3. Tea research and application center of Recep Tayyip Erdoğan University**

This center was established in 2017. This center's mission is to conduct tea research on a national and worldwide scale. Participation and organization of national and worldwide congresses are other priorities.

## **7.2.2. Tea Breeding Facilities in Türkiye**

Initially, tea breeding effort was assigned to Çaykur as a mission. It could be stated that clonal selection research have been conducted in Türkiye, as discussed in detail below.

### **7.2.2.1. Clonal selection**

Clones are genetically identical clones of the source plant. Multiple objectives include equal yield, quality, and pathogen resistance, among others. On the other hand, the existing genetically diverse tea genotypes are not uniform in terms of quality. According to Bandyopadhyay and Das (2008), such populations are less affected by environmental factors.

Since the 1940s, ÇAYKUR has enhanced tea clones and distributed those to farmers. Farmers quickly removed the established plantations and replaced them with traditional crops such as maize. However, farmers noticed that after a short time, this plant has a great financial potential, and a tea establishment arose. However, seeds were used, and because to the cross-pollinating nature of the tea plant, great astonishing variation emerged in Turkish tea plantations. This provides a solid foundation for clonal tea selection. Large genetic variation is required in selecting genotypes resistant to diseases and pests, and these characteristics are critical in adapting to a changing environment (Barua, 1963; Wachira et al., 1995). Before replanting tea plantations in Türkiye with new, high-yielding genotypes, it is vital to cultivate and protect existing, diverse populations. Various promising tea clones, including ‘Tuglali-10’, ‘Derepazari-7’, and ‘Pazar-20’ have been

identified firstly (Öksüz, 1987). Muradiye, Gündoğdu, Fener3, Enstitü1, Enstitü2, Hamzabey, Hayrat, Çayeli, Ardeşen, Fındıklı, Pazar and İyidere were results of following selection work. These clonal selection process was achieved primarily by ÇAYKUR in the region. The list of clones is given in Table 2.

**Table 2.** Selected clones by the Tea Research Institute in Rize/Türkiye\*

Name	Province of origin	Selected by
Ardeşen	Rize	RTRI
Ali Rıza Erten	Rize	RTRI
Çayeli-46	Rize	RTRI
Çiftekevrek	Trabzon	RTRI
Derepazarı-7	Rize	RTRI
Derepazarı-32	Rize	RTRI
Enstitü-1	Rize	RTRI
Çaykur-1	Rize	RTRI
Çaykur-2	Rize	RTRI
Çaykur-3	Rize	RTRI
Çaykur-4	Rize	RTRI
Enstitü-2	Rize	RTRI
Enstitü-9	Rize	RTRI
Enstitü-61	Rize	RTRI
Fındıklı	Rize	RTRI
Fener-3	Rize	RTRI
Gündoğdu-3	Rize	RTRI
Gündoğdu-19	Rize	RTRI
Güneysu-26	Rize	RTRI
Hamzabey	Rize	RTRI
Fındıklı	Rize	RTRI
Fener-3	Rize	RTRI
Gündoğdu-3	Rize	RTRI
Gündoğdu-19	Rize	RTRI
Güneysu-26	Rize	RTRI
Hamzabey	Rize	RTRI
Hayrat	Trabzon	RTRI
İyidere	Rize	RTRI
Kalkandere-10	Rize	RTRI
Kalkandere-12	Rize	RTRI
Kömürcüler	Rize	RTRI
Kolhida	Trabzon	RTRI
Kömürcüler-1	Rize	RTRI
Kömürcüler-4	Rize	RTRI



Muradiye-10	Rize	RTRI
Of-25	Trabzon	RTRI
Of-37	Trabzon	RTRI
Of-53	Trabzon	RTRI
Of-66	Trabzon	RTRI
Of-264	Trabzon	RTRI
Pazar-14	Rize	RTRI
Pazar-20	Rize	RTRI
Pazar-42	Rize	RTRI
Sürmene-1	Trabzon	RTRI

**Table 2.** (continued)

Name	Province of origin	Selected by
Sürmene-6	Trabzon	RTRI
Sürmene-24	Trabzon	RTRI
Sürmene-29	Trabzon	RTRI
Sürmene-39	Trabzon	RTRI
Tuğlalı-10	Rize	RTRI
Üniversite	Rize	RTRI
Üniversite 2	Rize	RTRI

\*: Data was collected from ÇAYKUR; RTRI: Rize Tea Research Institute

### 7.2.2.2. Improvement of tea quality

A three years of Research Project namely “*Improvement of Tea Quality*” financed by DOKAP was signed in 2016 between the DOKAP and the Recep Tayyip Erdoğan University. During this Project quality analysis laboratory was established for the Faculty of Agriculture in Rize/Pazar. The characterization of biochemical, morphological, and molecular diversity heterogeneity in Turkish tea plantations was another objective. Finally, a small tea factory was built for the aforementioned Faculty of Recep Tayyip Erdoğan University.

### 7.2.2.3. Organic fertilizer application in tea production

-In 2016, a Research Project namely “Use of Organic Fertilizers in Rize Tea Plantations” carried out by the Faculty of Agriculture, Field Crops Department. This Project was funded by the Recep Tayyip

Erdoğın University. A number of 21 different organic fertilizers were tested at 8 locations to observe their effects on fresh leaf yield.

-A research project namely “Effect of Organic Fertilizers on Parameters with Medicinal value in Tea” supported by DOKAP was initiated after the first mentioned project. The organic tea production area in Hemşin was added to the aforementioned locations in this Project.

#### **7.2.2.4. Molecular marker work**

Plant breeding requires genetic variance, and before designing and launching plant improvement programs, the current genetic variability must be described using several approaches. Different molecular marker methods were employed by Kafkas et al. (2009) and Beriş et al. (2001, 2005, 2016) to determine genetic variation in chosen tea clones.

#### **7.2.2.5. Plant growth promoting bacteria as organic fertilizer**

Çakmakçı et al. (2012, 2013, 2016) reported valuable data about the possible use of plant growth promoting bacteria in tea.

#### **7.2.2.6. Tissue culture work**

In our former study (Yurteri et al., 2021), we aimed to exploit heterozygosity in immature embryos of the tea embryos through somatic embryogenesis to use the developing plantlets in breeding of tea. As a results of our study, increased kinetin concentrations combined with phenylboronic acid induced higher embryo survival rates, resulting in higher plant regeneration rates by somatic embryogenesis

from immature cotyledon tissues. The present results indicate that using tea cotyledons is an appropriate strategy for developing tea plants in vitro. Hence, it provides a new selection approach for producing new tea breeding lines. (Yurteri et al., 2021).

### **7.2.2.7. Important topics of tea cultivation in Türkiye**

#### **7.2.2.7.1. Renewal of tea plantations**

It is well known that tea plantations in Türkiye are widely from the seed origin. *Camellia sinensis*, on the other hand, is cross-pollinating, which has resulted in significant genetical variety in Turkish tea plantations. Furthermore, the development and harvest of tea plants varied, which posed challenges in tea processing due to unhomogeneous harvest of tea leaves. To solve this problem, new and high producing tea clones must be developed, followed by yield experiments at various conditions and altitudes. Cuttings or tissue culture techniques should be used to multiply the highest yielding clones. The construction of new tea plantations is a major challenge for the foreseeable future.

#### **7.2.2.7.2. Organic tea cultivation**

ÇAYKUR initiated organic tea farming facilities in the year 2003 Türkiye (Seyis et al., 2018). The locations Borçka/Artvin, Çamlıhemşin and Hemşin/ Rize were chosen as pilot areas. Further, ÇAYKUR established the “Organic Tea Farming Commission” for the organization of studies regarding organic tea farming and to create a road map for organic tea. Organic tea production increased from 378 da in 2007 up to 38,034 da in 2016. Also, a number of organic tea farmers

increased from 135 in 2007 up to 11,786 in 2016. The total quantity of purchased tea leaves, processed black and green tea have been increased from 2009 up to 2016. There is a remarkable increase in organic tea production in Türkiye during the last 20 years.

#### **7.2.2.7.3. Development of a national tea germplasm collection**

The presence, establishment, and description of plant genetic resources are all extremely valuable. Such genetic resources are critical for plant breeding programs in crop plants. The understanding of such material, as well as its current qualities and diversity, is critical to success. This is the base for use of these collections effective in breeding programmes (Kottawa-Arachchi, 2013).

As previously stated, Turkish tea plantations were multiplied through the using the seeds as production material. Large amounts of variation are now present in our tea plantations due to their cross-pollinating nature. This heterogeneity resulted in disparities in harvest time and, more importantly, biochemical composition. As a result, fresh high yielding clones must be selected in order to initiate new breeding attempts. In 2018 we declared that such a project will cover the next 50 years of tea breeding (Seyis et al., 2018). Now we are talking about a national tea germplasm collection about 2000 genotypes. This situation explains our foresight published in Seyis et al. (2018).

### **7.2.2.8. Possible development of alternative products in future**

#### **7.2.2.8.1. Tea seed oil**

Vegetable oils are major raw materials utilized in the chemical industry. With the rising expense of petroleum resources, as well as their finite and nonrenewable character, researchers' interest in the discovery and use of alternative renewable feedstock has been revived. Vegetable oils are a better alternative to petrochemicals since they are renewable, biodegradable, less harmful, and environmentally friendly (Van Broekhuizen, 2011). Tea seed oil (*Camellia sinensis*) is one such vegetable oil that has been neglected and exploited in large parts of the world. Tea seed oil is the oil produced from the seeds of *Camellia* species, both cultivated and wild, as well as other species. Though *C. sinensis* is primarily cultivated for commercial tea production, oil is rarely derived from this plant. Oil is extracted commercially from species such as *C. sasanqua*, *C. japonica*, *C. tenuifolia*, and *C. oleifera*. Seeds of *Camellia* species contain 20-70% oil, which is comparable in quality to olive (*Olea europaea*) oil (Table 3). As a result, it could be used as a substitute for olive oil and other edible oils. The tea seed oil is yellow in color, free flowing, has a pleasant odor, and may be stored at room temperature for three months without losing quality. (Roberts and De Silva, 1972). Fatty acid composition of *C. sinensis* seed oil consisted of 56% oleic acid, 22% linoleic acid, 21.5% palmitic acid, 2.9% stearic acid and 0.3% linolenic acid (Sahari et al., 2004; Rajaei et al., 2008). The major fatty acid (50% of the total oil) in the *C. sinensis* seed oil was oleic acid (Rajaei et al., 2005). Therefore, with regard to oleic acid, *C. sinensis* seed oil can be classify between sunflower and

olive oil (Sahari et al., 2004). The proportions of UFAs and SFAs in the extracted oils were 58.1–71.7% and 17.4–23.7%, respectively (Rajaei et al., 2005). This oil had little tendency to dry because of the low C18:2 and C18:3 contents that cause polymerization (Sahari et al., 2004).

**Table 3.** Oil content in camellia species and other crops\*

Species	Percent oil (dry weight basis)
<i>C. sasanqua</i>	60-70
<i>C. japonica</i>	66-70
<i>C. oleifera</i>	40-50
<i>C. tenuifolia</i>	40-50
<i>C. sinensis</i> (Common tea)	20-30
Groundnut	44-50
Olive (Common olive)	15-40
Sunflower	35-48
Mustard	30-48

\*: Ouwor et al. (1985); Kamenga (1985); Anonymous (1950)

#### 7.2.2.8.2. Tea honey

Recently several physiological functions (e.g. antimicrobial, antioxidant, antitumor and immunomodulatory activities) of tea flowers have been reported (Yoshikawa et al., 2005), and the flowers have received attention as a natural healthy material for food and cosmetics. The aromatic tea blooms contain sweet nectar, which is not widely known. Honeybees may be drawn to the tea nectar. According to one study of bee pollen obtained from tea plant blossoms, honeybees prefer tea pollen (*Camellia sinensis* L.) (Lin et al., 1993). Saito et al. (2015) obtained honey from tea flowers. They reported that the nectar of tea flowers is attractive to honeybee, but not toxic. Also researchers stated that their new findings presents significant information on the relationship of honeybees (*Apis mellifera* L.) and tea flowers, can

activate tea and beekeeping industry, leading to develop the production of honey from tea nectar.

#### **7.2.2.8.3. Tea from *Camellia sinensis* flowers**

*Camellia sinensis* flowers are also important in terms of a medicinal and aromatic plant. The flavonoids content in flowers of tea has been reported to be higher than the leaves (Yang et al., 2007). Tea flower also demonstrated strong antioxidant effects (Chen et al., 2012). Unfortunately, less attention has been given to flower of tea (*Camellia sinensis*). In fact, tea flower, as well as tea, has been considered a healthful beverage since ancient times, particularly in southern China (Chen et al., 2012). Tea flowers contain comparable chemical compositions as tea leaves. (Yung et al., 2003). Tea flowers contained many nutrition compounds, such as sugar, protein, vitamins, sucrose, amino acid, caffeine and tea polyphenols (Yang et al., 2007). As a result of this, it is possible to deduce that tea flowers have significant application value as leaves.

### **8. CONCLUSION**

Climate change will have a significant impact on future tea production, independent from the geographic distribution of the tea crop. Tea is already cultivated on suitable land and as a cash crop is historically the preferred choice for most suitable growing areas. Importantly, there are several uncertainties due to climate change impacts that are not yet fully understood but which could potentially affect future production levels. These include the frequency of natural

disasters; the proliferation of certain pests and diseases and higher infrastructure cost.

Tea (*Camellia sinensis* (O.) Kuntze) is the biggest income of farmers in the Northern Black Sea Region of Türkiye. Since the 1930s this perennial plant arised to a strategic important economic plant. In Türkiye, the tea production is concentrated more on black tea production with a small amount of green tea production. We assume that the tea plant will cover its importance in Türkiye also in future.

To make future plans for a special plant it is necessary to create a national germplam collection. In Türkiye, tea (*Camellia sinensis*) improvement is more concentrated on clonal selection, the first step of breeding work. As we know, the age of tea plantations in Türkiye is not well recorded and they are located in different cities and districts, displaying different climate and soil conditions. Therefore it will be not correct to use a direct comparison, because they will naturally display distinct characteristics.

All breeding programmes regarding tea should be directed to the described changes due to expected climate change on the world, which will effect also Türkiye. To have the right to comment on tea production and breeding on the World these issues has to be concerned. As described in detail, Türkiye has also the opportunity to develop new alternative products from the tea plant.



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## CHAPTER 10

### EFFECT OF ORGANIC FERTILIZERS ON YIELD AND QUALITY PARAMETERS IN *Camellia sinensis* (O.) Kuntze IN RİZE

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## **INTRODUCTION**

Organic agriculture can be defined as plant cultivation with non use of chemical/synthetic fertilizers and pesticides or genetically modified organisms, growth hormones, and antibiotics. Products originating organic farming are gaining popularity from day to day due to their nutritional and health benefits. Organic agriculture also protects the environment and has a greater socio-economic impact on a nation (Das et al., 2020).

Tea areas in Türkiye, displaying four climate characteristics, are in fallow up to six months. The reality that snow is falling on tea bushes in Türkiye, gives them them an extra significant feature. Consequently, there is no pesticide application Turkish tea plantations. Correspondingly, Turkish tea can be defined as “the most natural tea” compared with other teas in the World (Yurteri et al., 2019).

The tea production in Türkiye started more later compared with the other producer countries, but its production and related the industry developed very rapidly in in a relatively short time (Yurteri et al., 2019). In the 1950's tea production was under 25,000 tons, but in recent decades this value arised to significant quantities. Meanwhile, Türkiye holds a share of 3% between tea producer countries. According to the Food and Agriculture Organization (FAO) statistics, Türkiye ranks 8th place in the world production area of tea after China, India, Sri Lanka, Kenya, Indonesia, Vietnam and Myanmar. Regarding world tea production in the world Türkiye ranks at the 5th place after China, India, Kenya, Sri Lanka and Vietnam.



To feed the increasing human population the main aim has been the increase of yield of per unite area and this can be achieved in agriculture using fertilization. The kind of fertilizers used for this goal can be very different. The most known are organic and chemical fertilizers. If we talk about organic fertilizers we will remember farm manure which is natural and of organic character. Because chemical fertilizers are synthesized they can contain one or much more nutritive elements. If chemical fertilizers are used intensive negative effects could occur, which are determined by conducted experiments (Demiryürek, 2011).

In Türkiye tea production is mainly located in the cities Rize, Ordu, Giresun, Trabzon and Artvin at the Black Sea Region (Anonymous, 2015). In the world, tea production is located at the equatorial region or around it, but in Türkiye tea regions are located compared with the world at a top zone. In Asian countries tea production covers the whole year, but in our country where four climates can be met tea production is limited with six months because of snow in the winter period. Beginning from the first tea production in Türkiye our farmers did not use any pesticides in tea cultivation. The most important reason for this is that winter conditions decrease pests in natural mean. Correspondingly there existed no need for any use of pesticides. We know that in organic tea production any use of pesticides and chemical fertilizers is not allowed. Therefore, if Türkiye can switch to the use of organic fertilizers organic tea cultivation can be started and this a great advantage for Türkiye (Saklı, 2011).

## **1. HISTORY OF ORGANIC TEA PRODUCTION**

Organic agriculture is practiced in almost every country, and its share of agricultural land and farms is increasing everywhere. The organic product market is expanding rapidly, not only in Europe, Japan, and North America (the major markets), but also in many other countries, including many so-called developing countries. In many countries, the lack of state regulations for organic agriculture makes distinguishing organic from low-chemical or even non-organic products difficult. Many countries' governments are taking an interest in organic agriculture. On a global scale, the FAO is increasing its support for organic farming (Williges 2004). For high crop production, modern scientific technology encourages the use of chemical pesticides, herbicides, fungicides, and fertilizers. It causes the entire agricultural production to be chemicalized, causing the environment and soil to become unhealthy, all biodiversity and ecosystems to become ill, and the entire ecology to become chemicalized. Modern agriculture technology creates an organic farming system. Organic farming encompasses all aspects of farming, including fertilizer, soil management, plant or seed selection, irrigation, pest and disease management, and biological control methods. In that case, organic tea cultivation may benefit from direct pest control using natural pesticides (Khanal, 2012).

Organic agriculture is a production system that promotes soil, ecosystem, and human health. It is based on ecological processes, biodiversity, and cycles that are tailored to local conditions rather than

the use of harmful inputs. Organic tea farming is based on four principles: the health principle, the ecology principle, the fairness principle, and the care principle. Organic tea farming encourages and improves biodiversity, biological cycles, and soil biological activity through management practices that restore, maintain, and improve ecological harmony. The organic tea production system differs from the non-organic tea production system (Acharya, 2009).

The organic movement in Sri Lanka began in the 1980s as a result of local NGOs' contact and inspiration with the Philippine organic agriculture movement. In 1982, a group of local non-governmental organization (NGO) representatives, planters, scientists, and environmental officers drafted a Memorandum of Association to establish the Lanka Organic Agriculture Movement (LOAM). This is the official starting point for the spread of organic agriculture in Sri Lanka (Williges, 2004).

Türkiye's Organic Tea Production Parallel to global developments, ÇAYKUR began research in 2003 to increase organic tea farming in our country. Borçka/Artvin, Çamlıhemşin, and Hemşin/Rize were chosen as organic tea production areas in the context of organic tea farming. ÇAYKUR established the "Organic Tea Farming Commission" in 2006 to organize studies on organic tea farming and production and to develop a road map for organic tea. In 2006, "Farmer Briefing Meetings" were held to inform farmers about the advantages and disadvantages of organic tea farming. To begin the

organic tea production project, a contract with 135 farmers covering 37,8 ha was signed in 2007.

Organic farming developed and is rapidly developing in Türkiye as a result of related and emerging problems with chemical fertilization. The first reason is the increased demand for organic tea products, particularly from other countries. The Ministry of Agriculture, Food, and Livestock, as well as related universities, research firms, and non-governmental organizations, are all looking into this issue. Furthermore, the bias against chemical fertilizers sparked interest among local farmers and public opinion, leading to the establishment of local markets, among other things (Aksoy and Altındışlı, 1999; Kenanolu and Karahan, 2002; Demiryürek et al., 2008).

Organic fertilizer studies were initiated in collaboration with ÇAYKUR in the Black Sea Region to investigate the effect of organic fertilizers on tea yield and quality parameters from 2017 to 2019. The first two years of the project (2017-2018) were funded by the Recep Tayyip Erdoğan University Scientific Research Unit (22.749,99 TL), and the years 2018-2019 were funded by DOKAP with a budget of 1.650.000 TL.

## **2. CATECHINES IN TEA (*Camellia sinensis* L.)**

Tea, a product derived from the leaves and buds of the plant *Camellia sinensis*, is one of the richest catechin sources and contains, as the main catechin, (-)-epigallocatechin-3-gallate (EGCG) (Figure 1), which has numerous health benefits including anticancer, anti-obesity, antidiabetic, anticardiovascular, anti-infectious, hepatoprotective, and

neuroprotective effects. A number of human epidemiological and clinical studies on tea have provided evidence for its anticancer benefits, which have been supported by cell-based and animal experiments, though studies with contradictory findings have also been reported. Furthermore, detailed molecular mechanisms for the action mechanisms of other catechins have been proposed (Isemura, 2019).

This study looked at the impact of organic fertilizers on tea yield and quality (*Camellia sinensis*).

### **3. MATERIAL AND METHODS**

Organic fertilizer trial was conducted during 2017-2019 in 8 different locations to investigate the effect of organic fertilizers on tea yield and quality parameters. About 23 organic fertilizers were used in the study in the first year, but only 9 organic fertilizers present in all three years will be presented here at the selected locations Ardeşen, Fındıklı and Hopa/Pınarlı.

#### **Used organic fertilizers:**

Control, Chemical (25-5-10), (1) Özra (Solid), (4) Süpersol (Liquid), (5) Süttaş (Solid), (7) Agrotolia (Solid), (8) Hexaferm (Solid), (11) Afyon Enerji (Solid), (12) Turkuaz (Liquid), (16) Eyogreen (Liquid) and (19) Yemmix (Liquid)

The randomized block design with three replications was used as experimental design and the conuction of locations was done as shown in Figure 1.



**Figure 1.** Conduction of field trials

**Yield:**

Fresh leaves were harvested from  $10\text{m}^2$  in every plot and weighted as  $\text{kg}/10\text{m}^2$  and calculated for  $\text{kg}/\text{da}$ .

**Total phenolic content:**

***Determination of total phenolic contents***

The UV-Vis spectrophotometer device was used to determine the total phenolic content ( $\text{mg GAE}/\text{gr DW}$ ) of investigated samples. The sample pretreatment was done as described in the FRAP method. Based on the method published by the International Organization for Standardization (ISO) 14502-1 gallic acid was used as standard. Tubes containing water involving Folin reagent was supplemented with Sample extract as  $1/10$  of the total volume and  $300\ \mu\text{l Na}_2\text{CO}_3$ . Afterwards, all tubes were kept in a ultrasonic shaker ( $50^\circ\text{C}$ ) for 15 min.

The measurement were done using a UV spectrophotometer device at a wave length of 765 nm to obtain the absorbance values (Yurteri et al., 2019).

### ***Antioxidant activity***

The antioxidant activity of collected samples was determined using a modified version of the FRAP assay described by Izzreen and Fadezelly (2013) as mg FeSO<sub>4</sub>/gr DW. As a pretreatment, 0.1 g of each dried sample was mixed with methanol (80%) to make a volume of 10 ml. The samples were mixed in a water bath (50 °C) for 20 minutes before being kept in the dark for 1 hour. After that, the mixture was centrifuged for 20 minutes at 4000 cycle/min to obtain the extracts, which were used to determine the phenolic content and antioxidant activity of the investigated samples. The collected samples were evaluated in terms of their antioxidant activity values.

Green tea leaves were collected during two shooting periods, dried in a drying oven at 40 °C, and their antioxidant activity was determined using a UV-spectrophotometer and the FRAP method. The FRAP method was used to determine the antioxidant capacity of the investigated samples (pretreatments completed). The FRAP method bases on the colourization after the degradation of the Fe<sup>+3</sup> ion, bounded to TPTZ in an acid environment, to Fe<sup>+2</sup>. 300 mM acetate buffer (pH 3.6), 10 mM 2.4.6-tripyridyl-s-triazine (TPTZ) and 20 mM FeCl<sub>3</sub>.6H<sub>2</sub>O solutions were mixed at a proportion of 10:1:1 as FRAP (ferric reducing / antioxidant power) reactives to obtain a buffer solution. To obtain a calibration curve, different standard probes were

prepared in a  $\text{FeSO}_4 \cdot \text{H}_2\text{O}$  solution. The final samples were obtained by mixing 1980 l FRAP dispersive with 20 l sample and then waiting 3 minutes in an ultrasonic shaker ( $50^\circ\text{C}$ ). The final absorbance values were obtained using a UV Spectrophotometer device at a wave length of 595 nm (Özcan et al., 2017; Yurteri et al., 2020).

### **Quality parameters:**

#### ***Preparation of plant samples for determination of phenolic compounds***

After weighing 3 g on a precision balance, all 432 samples in the study were placed in glass jars with lids and 100 ml ( $100^\circ\text{C}$ ) distilled water was added. The resulting mixtures were macerated every 15 minutes and left to infuse for 30 minutes. At the end of this period, the mixtures were filtered 3 times with the help of filter paper and liquid extracts were obtained and the working samples were taken into vials and made ready for analysis.

#### ***Determination of phenolic compounds***

The amounts of catechin, caffeine, epigallocatechin, epigallocatechin gallate and epicatechin in the extracts were determined using a high performance liquid chromatography (HPLC) device.

Stock solutions obtained from the standards of to be determined compounds mix solutions of 0.5-1-2.5-10-25-35 and 50 ppm concentrations were used to create calibration curves were created and the amount was calculated.

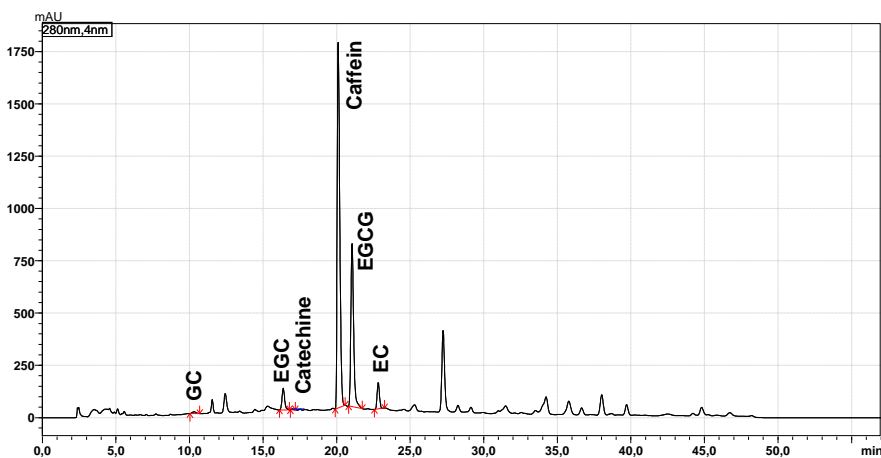


High pressure liquid chromatography (HPLC) operating conditions were as follows:

Device: Shimadzu LC-2030 C 3D model, Column: Purospher Star RP-18 5 $\mu$ m, 4.0x250mm (Merck);

Mobile phase A: Methanol, Mobile phase B: pure water, Detector: Diode Array Detector (DAD)

The HPLC Chromatogram of investigated compounds is given in Figure 2.



**Figure 2.** HPLC-Chromatogramme of investigated compounds

#### 4. RESULTS AND DISCUSSION

While this field trials were conducted over 8 locations, three selected locations – namely, Ardeşen, Fındıklı and Hopa/Pınarlı – will be discussed based on fresh leaf yield and antioxidant capacity, total phenol content, catechin and caffeine content.

#### 4.1. Fresh Leaf Yield (kg/da)

##### Ardeşen location:

Yield values obtained from Ardeşen Location can be seen in Table 1. Very significant differences were determined between applied fertilizer applications and years comparing obtained fresh yield values.

Compared with chemical fertilizer application organic fertilizers number 5 and 11 were located in the same group a regarding fresh tea yield, whereas fertilizer number 11 overranged chemical fertilizer application.

**Table 1.** Fresh yield values (kg/da) in Ardeşen location based on years (2017-2019) and applications\*

Applications	2017	2018	2019	Mean
Chemical	1481.7	1555.4	1462.0	1499.7 <sup>a</sup>
Control	1288.1	1083.1	725.9	1032.3 <sup>de</sup>
1	1395.5	1207.9	1058.1	1220.5 <sup>bcd</sup>
4	1329.1	1194.4	964.3	1162.6 <sup>cde</sup>
5	1620.6	1733.1	986.1	1446.6 <sup>ab</sup>
7	1364.9	1345.2	728.8	1146.3 <sup>de</sup>
8	1677.7	1335.2	1247.6	1420.2 <sup>abc</sup>
11	1965.9	1702.1	1096.9	1588.3 <sup>a</sup>
12	1164.9	1064.9	891.0	1040.2 <sup>de</sup>
16	1324.3	1219.3	1074.3	1205.9 <sup>bcd</sup>
19	962.9	919.1	871.0	917.7 <sup>e</sup>
Mean	1415.9 <sup>a</sup>	1305.4 <sup>a</sup>	1009.6 <sup>b</sup>	

\*: There is no difference between values marked with the same capital letter

##### Fındıklı location:

Three-year (2017-2018 -2019) fresh leaf yield values of the fertilizers applied to the tea plant in the Fındıklı location are given in Table 2. Average fresh leaf yield values in tea plant in terms of years

varied between 1930.8-1362.8 kg/da. The highest fresh leaf yield was obtained in 2018, followed by 2019 (1586.8 kg/da) and 2017 (1362.8 kg/da), respectively (Table 2).

When the average values of fertilizer applications are examined; although there was a numerical difference between 5 (2193.0 kg/da) and 8 (1974.8 kg/da) applications in terms of fresh leaf yield, they were statistically in the same group. The lowest fresh leaf yield was obtained from the application of fertilizer number 16 with 1201.0 kg/da.

**Table 2.** Fresh yield values (kg/da) in Fındıklı location based on years (2017-2019) and applications\*

Applications	2017	2018	2019	Mean
Chemical	1602.2	2267.5	1778.4	1882.7 <sup>bc</sup>
Control	1144.0	1614.0	1122.5	1293.5 <sup>d</sup>
1	1416.5	2158.8	1771.9	1782.4 <sup>bc</sup>
4	1031.1	1601.2	1504.4	1378.9 <sup>d</sup>
5	1647.9	2646.1	2285.0	2193.0 <sup>a</sup>
7	1383.7	2033.0	1579.6	1665.4 <sup>c</sup>
8	1609.1	2232.9	2082.3	1974.8 <sup>ab</sup>
11	1562.1	2093.3	1757.6	1804.3 <sup>bc</sup>
12	1219.5	1593.1	1238.7	1350.47 <sup>d</sup>
16	1160.0	1422.0	1021.0	1201.0 <sup>d</sup>
19	1214.9	1577.5	1313.7	1368.7 <sup>d</sup>
Mean	1362.8 <sup>c</sup>	1930.8 <sup>a</sup>	1586.8 <sup>b</sup>	

\*: There is no difference between values marked with the same capital letter

### Hopa/Pınarlı location:

The average fresh leaf yield values in terms of years in the tea plant varied between 1624.1-1144.9 kg/da. The highest fresh leaf yield was obtained in 2018 (1624.1 kg/da), followed by 2019 (1240.9 kg/da) and 2017 (1144.9 kg/da) respectively (Table 3). When the average

values of fertilizer applications are examined; in terms of fresh leaf yield, although there was a numerical difference between Chemical (1975.1 kg/da), 11 (1799.4 kg/da) and 8 (1775.5 kg/da) applications, they were statistically in the same group. The lowest fresh leaf yield was obtained from the application of fertilizer number 19 with 903.6 kg/da. Considering the application-year interaction, the fresh leaf yield varied between 2267.6-727.9 kg/da. The highest fresh leaf yield was obtained from the fertilizer application number 11 (2267.6 kg/da) in 2018 and statistically in the same group, 2018 chemical (2237.5 kg/da), 8 (2126.6 kg/da), 5 (2081.8 kg/da), chemical (2128.4 kg/da) applications were followed in 2019. The lowest yield value was obtained from the fertilizer application number 19 (727.9 kg/da) in 2019, and this trend was followed by the fertilizer application number 12 (732.4 kg/da) in 2019 (Table 3).

**Table 3.** Fresh yield values (kg/da) in Hopa location based on years (2017-2019) and applications\*

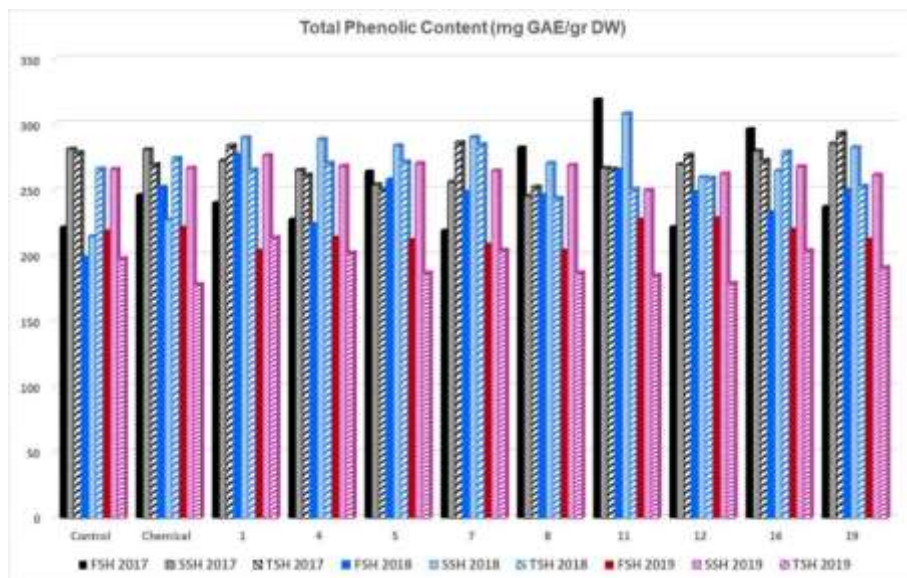
Applications	2017	2018	2019	Mean
Chemical	1559.3 <sup>def</sup>	2237.5 <sup>a</sup>	2128.4 <sup>ab</sup>	1975.1 <sup>a</sup>
Control	953.7 <sup>l-p</sup>	1218.4 <sup>h-k</sup>	935.4 <sup>l-op</sup>	1035.8 <sup>ef</sup>
1	1208.9 <sup>ijk</sup>	1903.7 <sup>bc</sup>	1358.5 <sup>f-i</sup>	1490.3 <sup>cd</sup>
4	847.8 <sup>op</sup>	1113.3 <sup>j-m</sup>	890.8 <sup>m-p</sup>	950.6 <sup>f</sup>
5	1309.7 <sup>g-j</sup>	2081.8 <sup>ab</sup>	1541.4 <sup>d-g</sup>	1644.3 <sup>bc</sup>
7	1153.4 <sup>i-l</sup>	1518.9 <sup>d-g</sup>	1070.6 <sup>k-o</sup>	1247.6 <sup>de</sup>
8	1449.4 <sup>e-h</sup>	2126.6 <sup>ab</sup>	1750.5 <sup>cd</sup>	1775.5 <sup>ab</sup>
11	1480.2 <sup>efg</sup>	2267.6 <sup>a</sup>	1650.5 <sup>de</sup>	1799.4 <sup>ab</sup>
12	924.8 <sup>l-p</sup>	1085.0 <sup>j-n</sup>	732.4 <sup>p</sup>	914.1 <sup>f</sup>
16	915.7 <sup>m-p</sup>	1121.2 <sup>j-m</sup>	864.2 <sup>nop</sup>	967.0 <sup>f</sup>
19	791.4 <sup>p</sup>	1191.6 <sup>jk</sup>	727.9 <sup>p</sup>	903.6 <sup>f</sup>
Mean	1144.9 <sup>b</sup>	1624.1 <sup>a</sup>	1240.9 <sup>b</sup>	

\*: There is no difference between values marked with the same capital letter

## 4.2. Total Phenolic Content

### Ardeşen location:

When the total phenolic content of Ardeşen location is examined (Figure 3); while the highest value was obtained from the first shoot harvest of application number 11 in 2017, the lowest values were obtained from the third shoot harvests of 2019. When the data of the harvest periods in three years are compared; while the total phenolic content was found to be highest in the second shoot harvest, the lowest value was determined in the third shoot harvest.

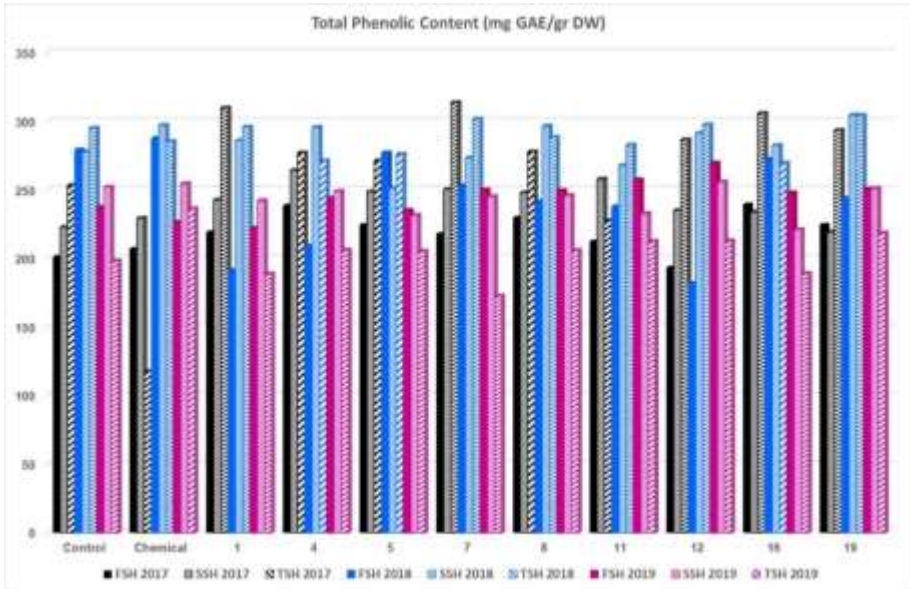


**Figure 3.** Total Phenolic Content at Ardeşen Location (First Shoot Harvest (FSH), Second Shoot Harvest (SSH), Third Shoot Harvest (TSH))

### Fındıklı location:

Phenolic content values at Fındıklı location changed over years and depending on organic fertilizer applications (Figure 4). While the highest value was obtained from the first shoot harvest of application

number 11 in 2017, the lowest values were obtained from the third shoot harvest of 2019. When the data of the harvest periods in three years are compared; While the total phenolic content was found to be highest in the second shoot harvest, the lowest value was recorded in the third shoot harvest.

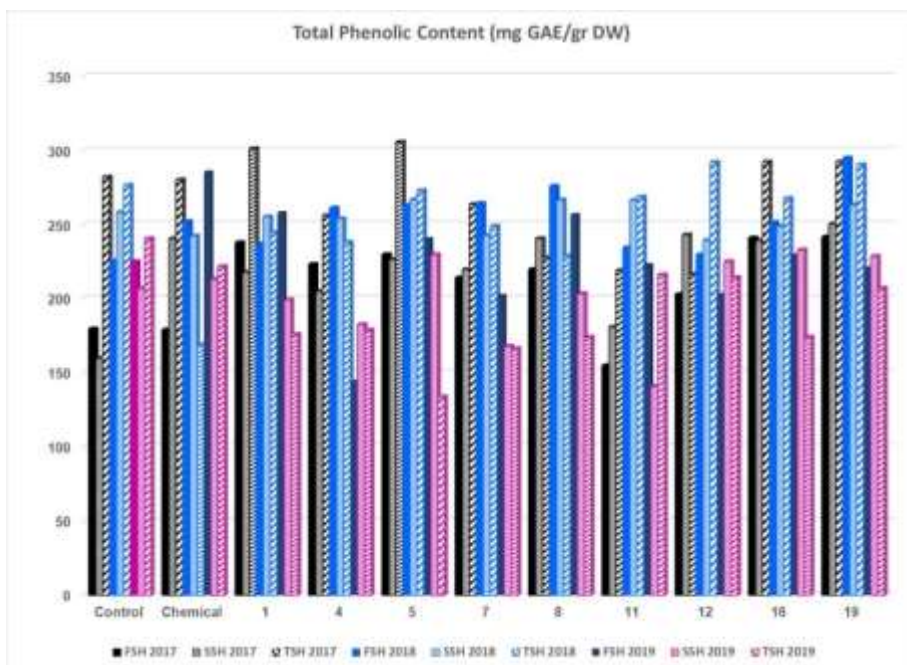


**Figure 4.** Total Phenolic Content at Ardeşen Location (First Shoot Harvest (FSH), Second Shoot Harvest (SSH), Third Shoot Harvest (TSH))

### **Hopa/Pınarlı location:**

When the total phenolic content of Hopa/Pınarlı location is examined; while the highest value was obtained from the third shoot harvest of application number 5 in 2017, the lowest value was obtained from the third shoot harvest of application number 5 in 2019 (Figure 5). When the data of the harvest periods in three years are compared; In general, in 2017 and 2018, the highest total phenolic content was found in the third exile period, the lowest data were reached in the first exile

period, and the lowest total phenolic contents in 2019 were determined in the third exile period.

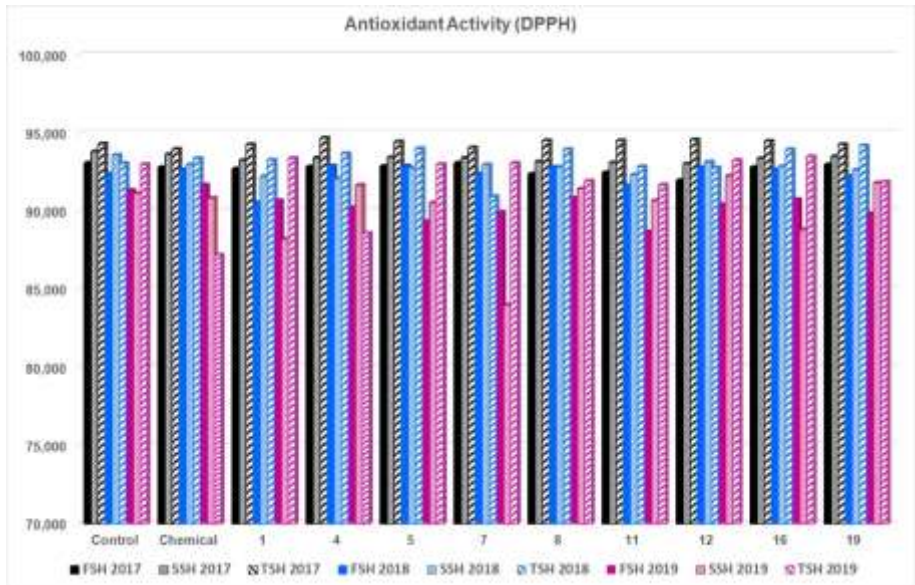


**Figure 5.** Total Phenolic Content at Hopa/Pınarlı Location (First Shoot Harvest (FSH), Second Shoot Harvest (SSH), Third Shoot Harvest (TSH))

### 4.3. Antioxidant Activity (DPPH)

#### Ardeşen location:

The highest antioxidant values were obtained from the 3rd shoot harvest in 2017 and the lowest value was obtained from the second shoot harvest of fertilizer number 7 in 2019 (Figure 6). When the data of the harvest periods in three years are compared; the antioxidant activity content was generally highest in the third harvest and the lowest value were obtained in the first shoot harvest.

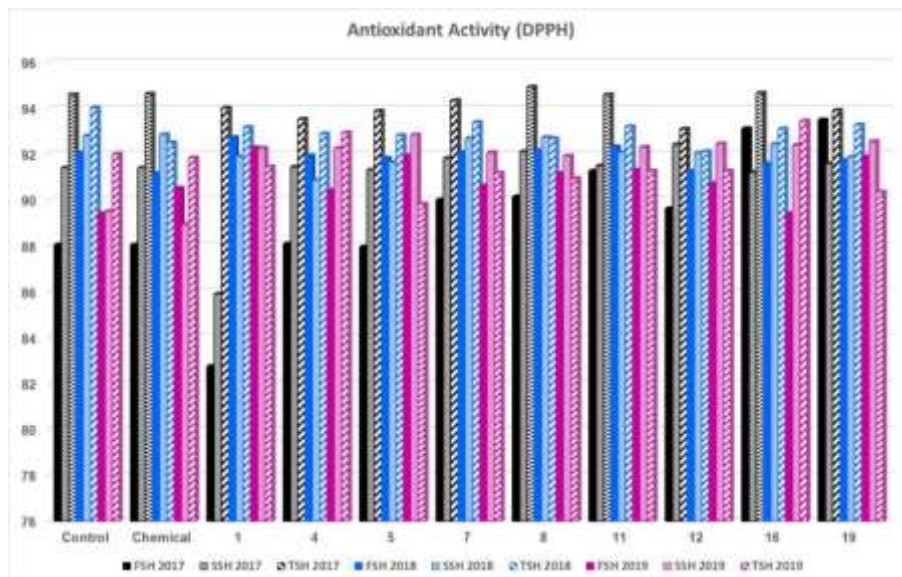


**Figure 6.** Antioxidant Activity at Ardeşen Location (First Shoot Harvest (FSH), Second Shoot Harvest (SSH), Thirth Shoot Harvest (TSH))

### **Fındıklı location:**

The antioxidant activity results of the Fındıklı location showed that highest antioxidant activity value was obtained from the 3rd shoot harvest of the organic fertilizer number 8 in 2017 and the lowest value was obtained from the first shoot harvest of organic fertilizer number 1 in 2017 (Figure 7). When the data of the harvest periods in three years are compared; in general, while the highest antioxidant activity content was determined in the third shoot harvest in 2017 and 2018, and in the second shoot harvest in 2019, the lowest antioxidant activity values were obtained in the first shoot harvest.

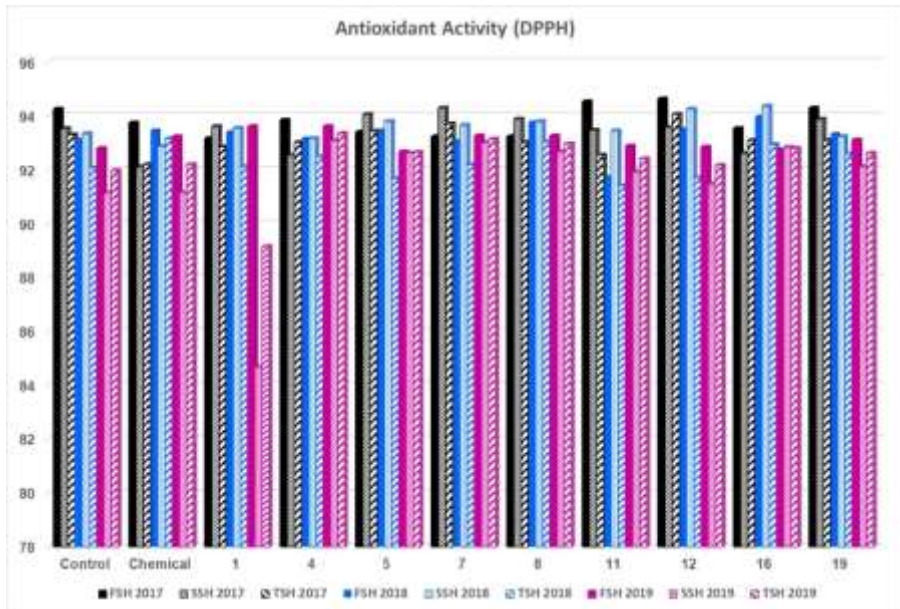




**Figure 7.** Antioxidant Activity at Fındıklı Location (First Shoot Harvest (FSH), Second Shoot Harvest (SSH), Third Shoot Harvest (TSH))

### **Hopa/Pınarlı location:**

At the Hopa/Pınarlı location the highest values were obtained from the first shoot harvest in 2017, the lowest value was obtained from the second shoot harvest with organic fertilizer number 1 in 2019 (Figure 8). When the data of the harvest periods in three years are compared; In general, the highest antioxidant activity content was reached in the first harvest time in 2017 and 2019, and in the second harvest time in 2018, while the lowest results were achieved in the third harvest time in 2017 and 2018, and in the second harvest time in 2019.



**Figure 8.** Antioxidant Activity at Hopa/Pınarlı Location (First Shoot Harvest (FSH), Second Shoot Harvest (SSH), Third Shoot Harvest (TSH))

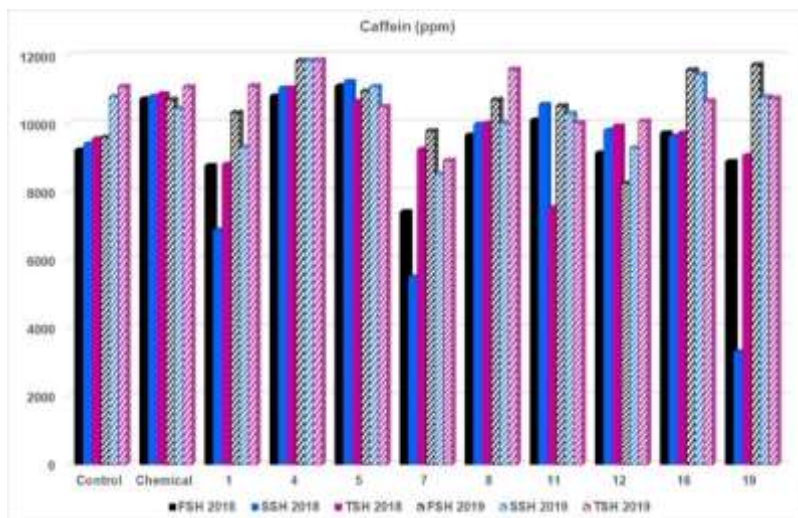
#### 4.4. Quality Parameters

The caffein, catechine, galloocatechine, epigallocatechine, epigallocatechinegallat and epicatechine content values of investigated samples over 3 harvest times are given for 2018 and 2019 only for the Fındıklı location, though in all locations a remarkable effect of applied fertilizers could be obtained (Figure 9).

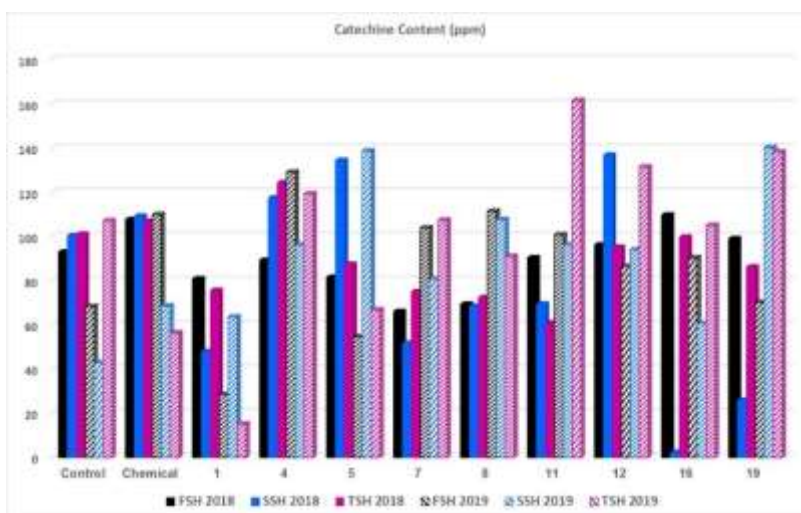
As can be seen in Figure 9 the caffein content of harvested samples from organic fertilizer applied parcels over 2 years and 3 harvest times. In 2019 the caffein values were higher in all 3 harvest times compared with 2018.

The catechine content changed from year to year and also based on harvest times. Different organic fertilizers showed remarkable effect

on the catechine contnet in both years. The highest catechine value was obtained from organic fertilizer number 11 at the 3. harvest time (Figure 10).

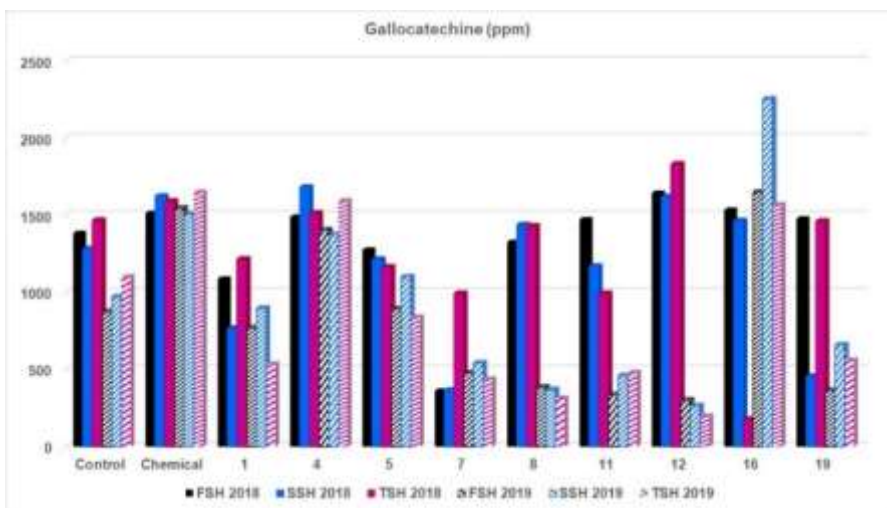


**Figure 9.** Caffein content values from organic fertilizer applied samples



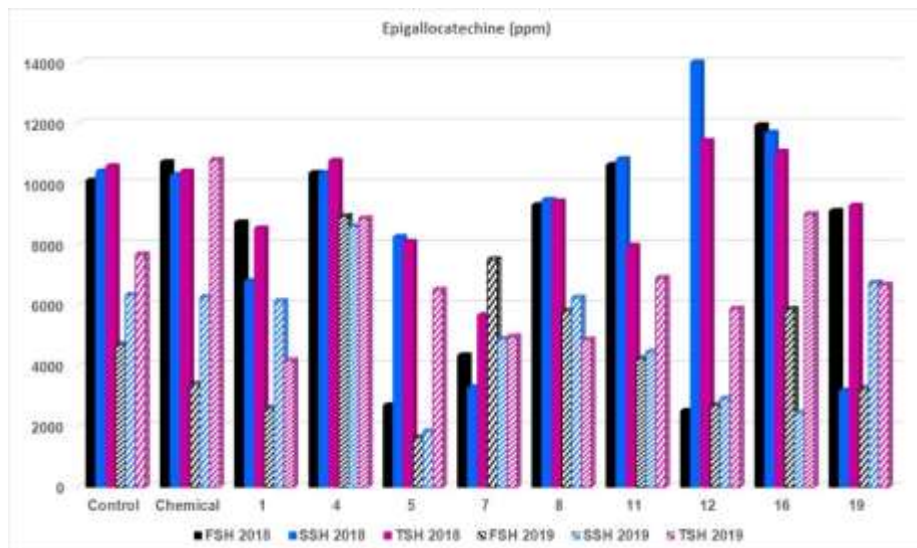
**Figure 10.** Catechine content values from organic fertilizer applied samples

The same situation could be observed regarding the gallicocatechine content (Figure 11). Some organic fertilizers (1 and 7) reduced the gallicocatechine content in both years and harvest times compared with chemical fertilizer application. The highest gallicocatechine contents were observed in application number 16 in 2019 and all three harvest times.



**Figure 11.** Gallicocatechine content values from organic fertilizer applied samples

Epigallocatechine content of analyzed samples changed in both years and all harvest times. Compared with chemical fertilizer application the highest epigallocatechine contents were determined in fertilizer application number 16 in 2018 (Figure 12). Generally epigallocatechine content of investigated samples differed between years and harvest times.

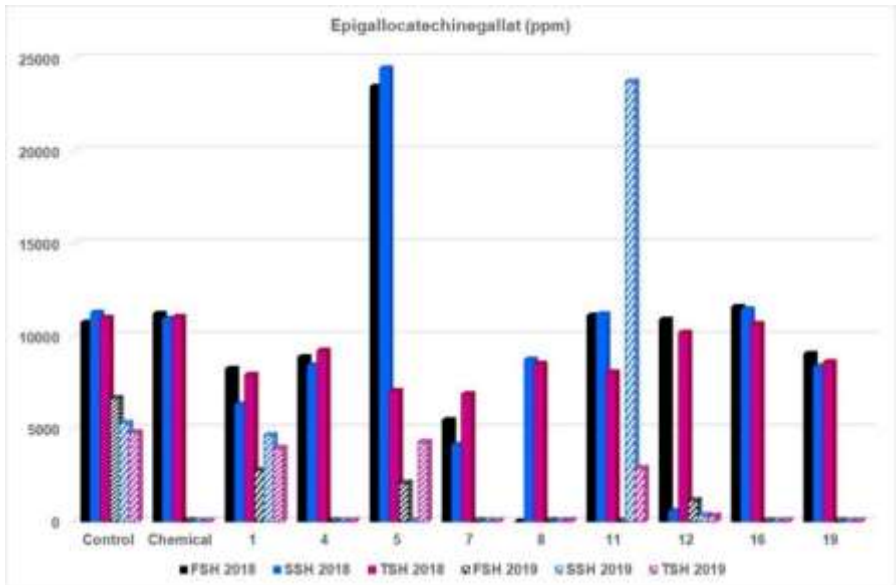


**Figure 12.** Epigallocatechin content values from organic fertilizer applied samples

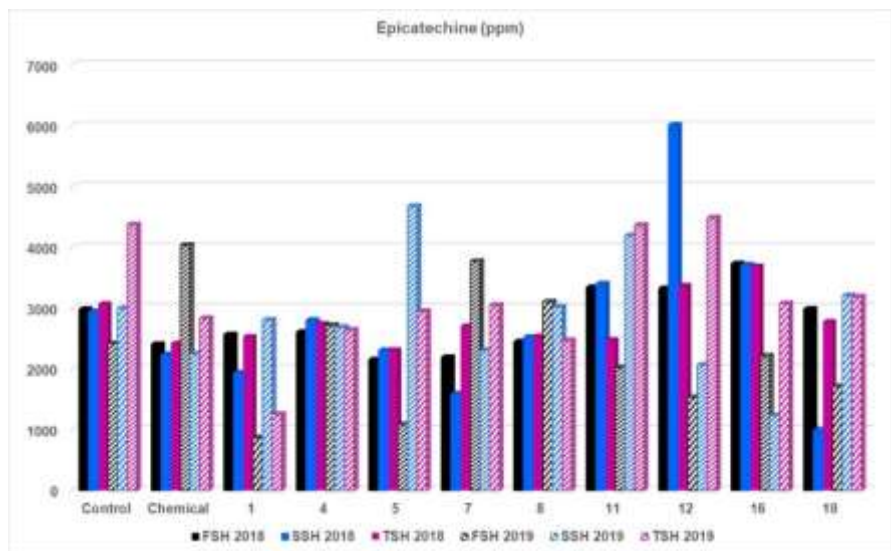
The epigallocatechin values differed between years and harvest times. This compound was the most effected compound between investigated quality parameters (Figure 13).

Epicatechin content of analyzed samples changed in both years and all harvest times. Compared with chemical fertilizer application the highest epicatechin contents were determined in fertilizer application number 5 in 2018 (Figure 14). Generally epicatechin content of investigated samples differed between years and harvest times.





**Figure 13.** Epigallocatechin gallate content values from organic fertilizer applied samples



**Figure 14.** Epicatechine content values from organic fertilizer applied samples

Under favorable temperature, rainfall, relative humidity, and evaporation conditions, tea yield is increased by nitrogen nutrition without having any adverse effects of large amounts of nitrogen supply (Hajiboland, 2017).

Tea plants require a lot of micro and macro nutrients to grow. A lack of these nutrients could have a negative impact on yield and quality. These nutrients are essential, and a lack of them or an insufficient supply will eventually result in poor seedling establishment and performance. In fact, the tea plant requires a lot of N, P, K, and Mg to grow (Islam et al., 2017). Plants require adequate nutrients to achieve maximum growth and productivity. Fertilizer use is critical in agriculture to increase yield and influence soil properties. So, when it comes to tea cultivation, fertilizer application is a standard management practice that significantly improves tea yield, productivity, and tea quality (Qiu et al., 2014).

The mean yield of tea plantations is about 1500 kg/da. Especially in the second and third year of conducted trials fresh leaf yields near the yield obtained from chemical fertilizer application could be achieved in all three locations. Three year results showed that is possible to use different kind of organic fertilizers in the context of a switch to organic tea production in Türkiye.

Several studies have been conducted to investigate the effects of nitrogen on tea quality. While nitrogen use improved green tea quality (Takeo, 1979), increasing nitrogen rates degraded black tea quality (Cloughley, 1983; Clowes and Mitini-Nkhoma, 1987; Malenga, 1987;

Owuor et al.,1987; Ellis et al., 1979; Cloughley, et al., 1983; Khando, 1989).

The quality of tea is dependent, in the first instance, on the chemical composition of the harvested shoots, and in the second instance, on the way in which they are handled, processed and stored (Yao et al., 2005). Many factors, such as geographical location, cultivar species, season, age of the leaves, climate and agricultural practices (soil, water, minerals, fertilizers) may influence the chemical composition of tea leaves (Saito et al., 2007; Jayasekera et al., 2011; von Staszewski et al. 2011; Kerio et al., 2012).

Amino acids, tea polyphenols, and caffeine are key elements in determining both taste and quality of tea (Lin et al., 2019). Cloughley (1983) stated the influence of genetic constitution, climatic conditions, fertiliser application and harvesting policy on caffeine levels.

Tea polyphenols have attracted huge interest because of their presumed associated health properties (Khan et al., 2007; Li et al., 2019). Numerous studies have indicated in earlier times that catechins and other polyphenols in tea exhibit powerful antioxidant activities (Dufresne and Farnworth, 2001).

The main polyphenol components of fresh and GT leaves are catechins which include epicatechin (EC), epigallocatechin (EGC), epicatechingallate (ECG), epigallocatechingallate (EGCG) and catechin (C) (Hour et al., 1999). Tea polyphenols are strongly influenced by various factors such as variations in leaf variety,



harvesting season, climate, processing method and analytical method (Luximon-Ramma, 2005; Gramza and Korczak, 2005; Zhuo et al., 2002; Wheeler and Wheeler, 2004).

Based on three location results we can clearly state that the application of organic fertilizers have a remarkable effect on the antioxidant activity, total phenol content, caffeine, C, GC, EC, EGC and EGCG content of harvested tea leaves.

## **5. CONCLUSION**

Tea (*Camellia sinensis* (O.) Kuntze) is the biggest income of farmers in the Northern Black Sea Region of Türkiye. Since the 1930s this perennial plant arised to a strategic important economic plant. In Türkiye, the tea production is concentrated more on black tea production with a small amount of green tea production. We assume that the tea plant will cover its importance in Türkiye also in future.

Currently, chemical fertilizer is the main fertilizer used in most of the countries because of easy to handle, ability to determine the exact amount of nutrient provided, small amount of fertilizer needed for nutrient provided. Chemical fertilizer application is beneficial for improving tea yield; however, excessive application limits fertilizer utilization and results in nutrient waste. As a result, tea planting revenue decreases. Furthermore, excessive fertilizer use can be harmful to the environment, resulting in issues such as soil acidity. Pests and diseases become more difficult to control as they become resistant to chemical fertilizers. In addition to, chemical fertilizers left in the soil for an

extended period of time can enter the food chain, where they build up in the bodies of animals and humans, creating health issues.

In 2018 ÇAYKUR planned to switch to organic tea farming in all tea plantations. But up to that time the recommendation and use of organic fertilizers lacked. If we consider that the amount of tea plantations are about 820.000 da the necessary amount of organic fertilizers are amazing. Therefore we need a very organized plan regarding the kind of organic fertilizer and its use in Turkish tea plantations.

Present results indicated that used organic fertilizer effected tea yield very significantly in investigated locations. Also investigated quality characteristics were effected from organic fertilizer applications compared with chemical fertilizer. The results has shown that the use of organic fertilizers can led to high yields as well as obtained with chemical fertilizer application in investigated locations.

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## CHAPTER 11

### **SALVIA TAXA REGISTERED IN YOZGAT FLORA AND THEIR BIOLOGICAL ACTIVITIES**

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## INTRODUCTION

The Lamiaceae family, which spreads throughout the world, consists of essential oil-producing herbaceous plants, shrubs, and rarely trees. Members of this family are important in the pharmacology and perfumery industry because they contain essential oils. These species are also used as spice and tea and are grown as ornamental plants. Many of them are of considerable economic importance.

Sage, which is a member of this family, is the general name of the species included in the genus *Salvia*. The Flora of Türkiye has 114 taxa, 58 of which are endemic, of the genus *Salvia*, which is stated to have over 1000 species worldwide (Wei et al., 2015, Güner et al., 2012).

There are most fragrant, mostly perennial species, as well as biennial and annual species. Its flowers are white, yellow, pink, blue, or purple. The genus *Salvia* is distributed in both hemispheres, especially in the tropics and subtropics, around the Mediterranean Sea and in Central Europe, and it spreads up to 1500 m above sea level in Mediterranean countries (Arslan et al., 2015).

Although leaves, shoot tips, flowers, and partly stems of *Salvia* species, which have been important medicinal plants since ancient times are used, the most utilized part is the leaves. Since *Salvia* species appeal to a wide consumer group (food industry, pharmaceutical and chemical industry, herbalists selling as retail products) due to their characteristics, their market potential is quite high.



The ones with the highest commercial value are *S. officinalis* L., *S. fruticosa* Mill. (syn: *S. triloba* L.), *S. pomifera* L., *S. lavandulaefolia* Vahl. and *S. sclarea* L., *S. fruticosa* and *S. tomentosa* species are mostly collected in our country. *S. officinalis* is not found in the Flora of Türkiye (Baydar, 2013).

Although these types are generally consumed as a tea, they are used in frying and meat dishes in the kitchens of European countries (Seçkin, 2014). *Salvia* species are used in phytotherapy and folk medicine for different purposes in the world and in our country. In general, *Salvia* species have been observed to exhibit a wide range of biological effects (antibacterial, antifungal, antiviral, antiseptic, analgesic, antioxidant, astringent, antispasmodic, hallucinogenic, central nervous system depressant, antisudorific, emanagogue, antidiabetic, anticancer, tuberculostatic, cardiovascular and insecticide, etc.) (Karamanos, 2000).

In this study, the biological activities of the *Salvia* taxa in the Yozgat Flora and brief information about the taxa are also included.

## **1. SALVIA TAXA REGISTERED IN THE YOZGAT FLORA**

According to TÜBİVES (2016) records, there are 254 taxa in Yozgat province. 15 of these taxa are in the Lamiaceae family. 4 species (*S. freyniana*, *S. aethopis*, *S. yosgadensis*, *S. cyanescens*) and 1 subspecies (*S. candidissima* spp. *occidentalis*) of *Salvia* genus were recorded in Yozgat flora. However, within the scope of the projects we

have carried out, 13 *Salvia* taxa have been identified in Yozgat (Table 1). The distribution of these taxa in Türkiye is given in Figure 1.

**Table 1.** *Salvia* taxa in Yozgat Flora<sup>1</sup>

	<i>Salvia</i> taxa	Local name	Endemic
1	<i>S. freyniana</i> Bornm. ex Freyn*	Göl şalba	+
2	<i>S. cryptantha</i> Montbret & Aucher ex Benth Sin.: <i>S. absconditiflora</i> (Montbret & Aucher ex Benth.) Greuter & Burdet	Kara şalba	+
3	<i>S. sclarea</i> L.	Paskulak	-
4	<i>S. aethiopsis</i> L.	Habeş adaçayı	-
5	<i>S. ceratophylla</i> L.	Tarak şalba	-
6	<i>S. ekimiana</i> Celep & Doğan	Bey şalbası	+
7	<i>S. russellii</i> Benth.	Kurdeşik	-
8	<i>S. verticillata</i> L. subsp. <i>amasiaca</i> (Freyn & Bornm.)	Hart şalbası	-
9	<i>S. hypargeia</i> Fisch. & C.A. Mey.	Siyahot	+
10	<i>S. virgata</i> Jacq.	Fatmanaotu	-
11	<i>S. yosgadensis</i> Freyn & Bornm.	Bozok şalbası	+
12	<i>S. candidissima</i> Vahl. subsp. <i>occidentalis</i> Hedge	Akgalabor	-
13	<i>S. cyanescens</i> Boiss. & Balansa	Mor galabor	+

<sup>1</sup>Source: Yozgat Bozok University Scientific Research Projects Project No: 6602-ZF/17-87and Project No: 2015ZF/A171



**Figure 1.** Distribution of taxa in Yozgat Flora in Türkiye<sup>1</sup>

<sup>1</sup>Source: <https://bizimbitkiler.org.tr/yeni/demos/technical/>

A general view of *Salvia* taxa at the flowering stage is presented in Figure 2. Brief information about taxa:

**1- *S. freyniana* Bornm. ex Freyn:** It is a perennial, semi-shrub, and the plant height varies between 15-35 cm in the flowering period. The plant exhibits a horizontal-upright development. It is an unbranched or rarely branched species. Petals are lilac-blue and there is a white spot on the lower lip (Doğan et al., 2008).

**Flowering Time (month):** 6-6

**Habitat:** Sandy calcareous slopes

**Altitude (m) :**900-1082

**Threat Categories:** CR (Critically Endangered)

**2- *S. cryptantha* Montbret & Aucher ex Benth. , Syn.: *S.absconditiflora* (Montbret & Aucher ex Benth.) Greuter & Burdet:** The stems of this species are upright and can be up to 30 cm in length. They generally do not show branching. The color of the sepals is yellowish-green, rarely purplish. The color of the petals can vary from white to pink (Doğan et al., 2008).

**Flowering Time (month):** 5-7

**Habitat:** Rocky calcareous slopes, dry steppe, road and field edges

**Altitude (m):** 650-2500

**Threat Categories:** -

**3- *S. sclarea* L.:** They are biennial or perennial plants with very rough, upright, and rectangular stems. The plant can grow up to 100 cm. Dense branching was observed in the upper parts of the plant. It has

flowers ranging from pink to light purple or white. The leaves are petiolate, heart-shaped, and hairy. It is a common species in Türkiye (Doğan et al., 2008; Arslan et al., 2015).

**Flowering Time (month):** 5-8

**Habitat:** Rocky volcanic slopes, mixed deciduous and coniferous forests, clay beds, fields, and roadsides.

**Altitude (m):** 1-2000

**Threat Categories:** -

4- *S. aethiopsis* L.: They are biennial or perennial, herbaceous shrubs. Their upright growing stems, which can be 25-180 cm tall, are four-cornered and solidly built. Simple leaves are mostly at the base, and the upper parts of the plant are few leaves. The petals of the flowers are white in color. It can be easily distinguished from other species by its hairy thick stems and candelabra-shaped inflorescences. It is a plant that is completely dragged by the wind when it dries and breaks. This species can be seen frequently in almost every region except Southeastern Anatolia (Doğan et al., 2008; Arslan et al., 2015).

**Flowering Time (month):** 5-8

**Habitat:** Steppe, volcanic and limestone slopes, sandy slopes, fallow fields, dry meadows, roadsides.

**Altitude (m):** 1-2100

**Threat Categories:** -

5- *S. ceratophylla* L.: It is a very sticky lemon-yellow biennial herb. Stems are solid, 25-70 cm. The leaves are mostly located at the

base. Petioles are almost absent. The inflorescence is a diffuse paniculate, yellowish-green, and the corolla is cream or sulfur yellow (Doğan et al., 2008).

**Flowering Time (month):** 4-7

**Habitat:** Limestone, volcanic and chipped slopes, cornfields and fallow fields, vacant lots.

**Altitude (m):** 300-2250

**Threat Categories:** -

**6- *S. ekmiana* Celep & Doğan:** Perennial herbaceous plants. Stems range from horizontal to upright, between 10-40 cm. The inflorescence is highly branched candelabra or not. Petals are white and upper lip is lilac (Doğan et al., 2008)

**Flowering Time (month):** 6-6

**Habitat:** Alpine areas, *Pinus* open spaces.

**Altitude (m):** 1700-2100

**Threat Categories:** EN (Endangered)

**7- *S. russellii* Benth.:** It is an herbaceous perennial. Stems 20-60 cm, erect, usually simple and numerous, emerging from a woody taproot. Corolla is violet blue in color (Doğan et al., 2008).

**Flowering Time (month):** 5-7

**Habitat:** Rocky slopes, vacant cultivated fields in grassy meadows in *Quercus* scrub.

**Altitude (m):** 100-1600

**Threat Categories:** -

**8- *S. verticillata* L. subsp. *amasiaca* (Freyn & Bornm.):** It is a perennial herbaceous structure and its stems are upright or ascending. Plant height 15-50 cm, numerous and less branched above. Corolla is violet-blue, lilac, rarely white (Doğan et al., 2008)

**Flowering Time (month):** 5-9

**Habitat:** Rocky slopes, Stipa steppe, sandy beds, meadows, *Quercus* and *Pinus* woodlands, fields, and roadsides.

**Altitude (m):** 20-2300

**Threat Categories:** -

**9- *S. hypargeia* Fisch. & C.A. Mey:** Perennial, herbaceous. Their stems show an upright development. The plant is 25-60 cm tall. Generally, no branching is observed in the trunk. Simple leaves are mostly located at the base of the plant. The upper parts of the leaves are greenish and the lower parts are covered with white hairs. Corolla ranges in color from magenta to purplish blue (Doğan et al., 2008).

**Flowering Time (month):** 6-7

**Habitat:** Limestone slopes, *Pinus brutia* forest, fallow fields.

**Altitude (m):** 800-2000

**Threat Categories:** LC (Least Concern)

**10- *S. virgata* Jacq.:** It is a perennial herbaceous plant. The stems are upright, 30-100 cm tall, and have a highly branched structure from above. Leaves are simple, usually scattered on the stem, sometimes collected at the base, The flower is in the form of a compound panicle.

Petals are violet, blue, or mauve, rarely white. This species can be seen in almost all regions (Doğan et al., 2008; Arslan et al., 2015).

**Flowering Time (month):** 5-9

**Habitat:** Roadsides, field edges, pastures.

**Altitude (m):** 1-2300

**Threat Categories:** -

**11- *S. yosgadensis* Freyn & Bornm.:** It is a herbaceous perennial. The body is 13-40 cm, usually quite upright and four-cornered. Petals are white or mauve (Doğan et al., 2008).

**Flowering Time (month):** 5-6

**Habitat:** Oak open areas, stepic areas and limestone rocky and stony areas

**Altitude (m):** 800-1635

**Threat Categories:** LC (Least Concern)

**12- *S. candidissima* Vahl. subsp. *occidentalis* Hedge:** Perennial herbaceous structure. Stems 30-60 (-90) cm, erect, branching above. The petals are completely white. There are two subspecies of this species. Of these, subsp. *candidissima* flowers white, lower lip yellow; subsp. *occidentalis* flowers are completely white (Doğan et al., 2008).

**Flowering Time (month):** 5-9

**Habitat:** Rocky, volcanic and limestone slopes, field edges.

**Altitude (m):** 800-1900

**Threat Categories:** -

**13- *S. cyanescens* Boiss. & Balance:** It has a perennial, herbaceous structure, a four-cornered stem that grows 25-70 cm upright. The branching is in the upper parts of the plant, and the color of the corolla varies from lilac to violet. It is a very close species to *S. candidissima* but is easily distinguished from it by its lilac-colored calyx and corollas, thinner appearance, and smaller nutlets. Inflorescences of the samples collected from the Mediterranean region are more frequent and dark-colored, and as one goes towards North Anatolia, both the corolla lengths increase and the flower colors become paler (Doğan et al., 2008).

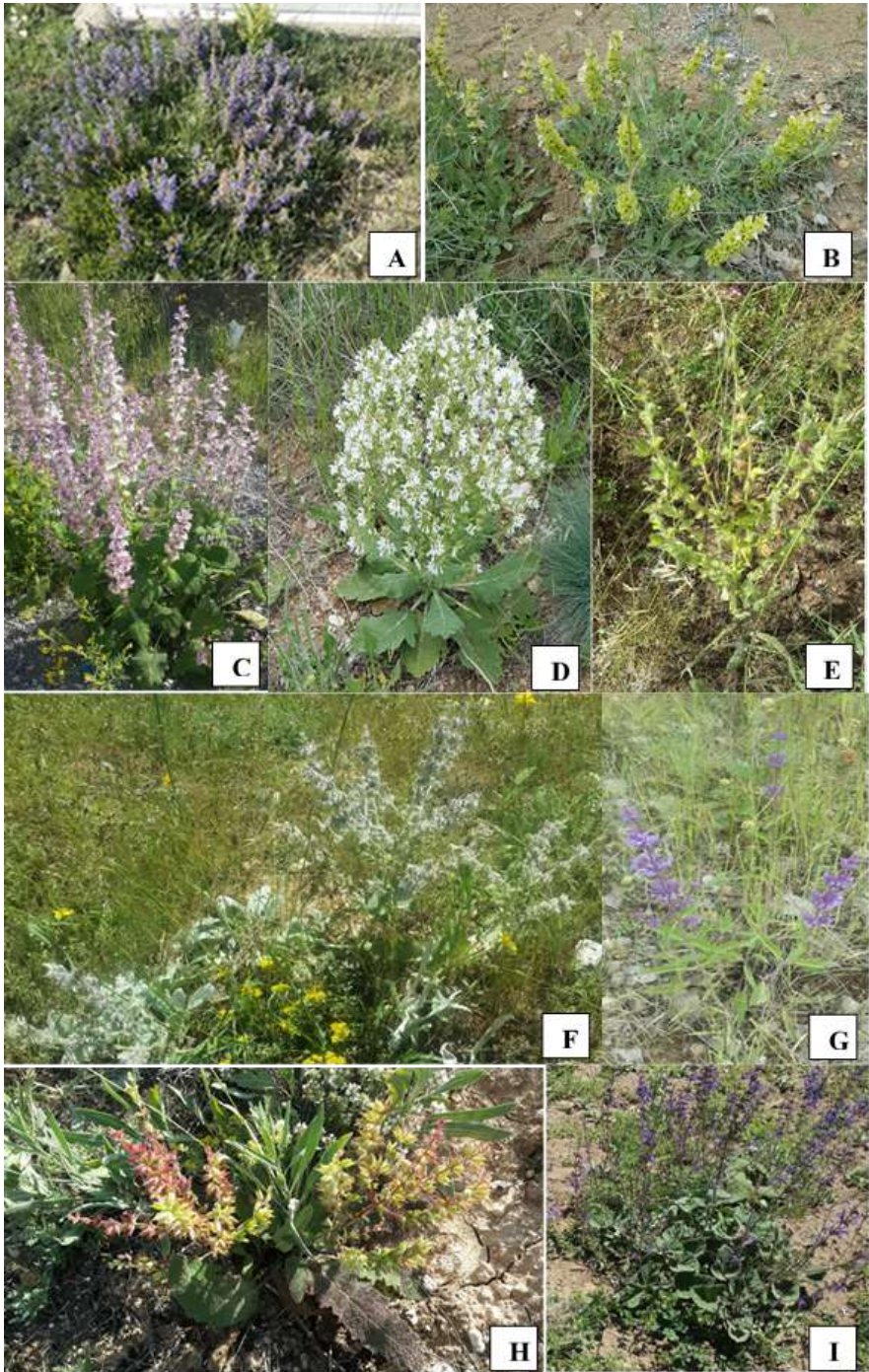
**Flowering Time (month):**6-9

**Habitat:** Limestone, serpentine and volcanic slopes, clayey slopes, *Pinus nigra* forest, gravel river beds, fallow fields, vineyards.

**Altitude (m):** 400-2300

**Threat Categories:** LC (Least Concern)





**Figure 2.** Images of the taxon in the flowering stage

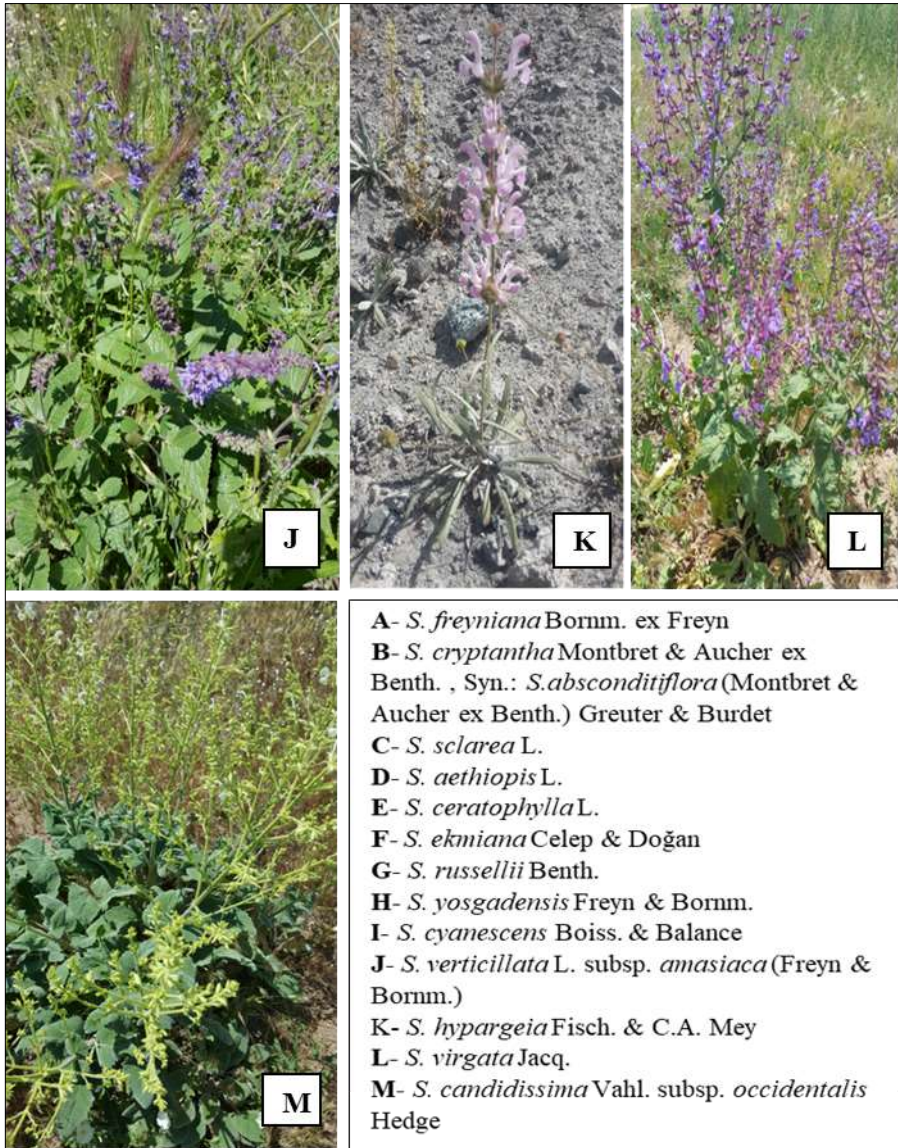


Figure 2. (Continued)

The economically evaluated parts of sage species are the leaves and flowers, and the extracts and essential oils obtained from these parts. The genus *Salvia*, which contains a large number of species and



is rich in terpenoids, flavonoids, and other phenolic substances, also exhibits diversity in terms of bioactive substances. However, there are many differences in pharmacological effects between species (Table 2). Many species of the genus *Salvia* are widely used in the preparation of traditional herbal medicines around the world.

In our country, most sage species are used in the form of dry leaves, mainly as tea and spice (Baydar, 2013). *Salvia* tea is traditionally used in the treatment of digestive and circulatory disorders, bronchitis, cough, asthma, pharyngitis, mouth and throat inflammation, depression, excessive sweating, skin problems, and many other diseases.

On the other hand, sage oil is an essential oil with a very strong antiseptic and antibiotic effect. For this reason, it uses as an additive to drugs made especially for throat infections, tooth inflammation, and mouth sores. Sage leaves and essential oil are used in folk medicine for many purposes such as soothing, analgesic, antiperspirant, expectorant, preventing cold and cough, relieving muscle pain, reducing high blood pressure, stomachic, and disinfectant. It is the most effective cold medicine. It is a natural antibiotic because of the effective substances it contains such as cineole (Baydar, 2013; Seçkin, 2014). Essential oils, alkaloids, glycosides, saponins, balms, waxes, resins, etc. secondary metabolites are the most basic products of industry (food, cosmetics, paint, medicine, etc.) directly or indirectly. *Salvia* species are especially rich in terpenoid compounds. Apart from these, they contain flavonoids, essential oils, and phenolic compounds (Baydar, 2013). Most medicinal

and aromatic plants rich in secondary metabolites have strong antioxidant effects. Sage species also have high antioxidant properties.

**Table 2.** Biological activities of *Salvia* taxa in Yozgat Flora

<i>Salvia</i> taxa	Biological activity	Reference
<i>S. freyniana</i> Bornm. ex Freyn*	Antioxidant	Topçu et al. (2017)
	Anticholinesterase	Topçu et al. (2017)
	Enzyme inhibitory	Topçu et al. (2017)
<i>S. cryptantha</i> Montbret & Aucher ex Benth.* Sin.: <i>S.</i> <i>absconditiflora</i> (Montbret & Aucher ex Benth.) Greuter & Burdet	Antimicrobial	Topçu et al. (2017)
	Anticholinesterase	Topçu et al. (2017)
	Antioxidant	Topçu et al. (2017)
<i>S. sclarea</i> L.	Cytotoxic	Topçu et al. (2017)
	Antimicrobial	Ovidi et al. (2021); Bisio et al. (2019); Küçük et al. (2019); Aćimović et al. (2018); Topçu et al. (2017); Kuźma et al. (2009)
	Antifungal	Aćimović et al. (2018); Topçu et al. (2017)
	Antioxidant	Ovidi et al. (2021); Aćimović et al. (2018); Topçu et al. (2017); Wu et al. (2012)
	Anticholinesterase	Topçu et al. (2017)
	Antibiotic resistance and antibiofilm activity	Wu et al. (2012)
	Cytotoxic	Bisio et al. (2019); Aćimović et al. (2018); Kuźma et al. (2009)
	Anti-inflammatory activity	Aćimović et al. (2018)
	Antibiofilm activity	Küçük et al. (2019)
	<i>S. aethiopsis</i> L.	Antioxidant activities
Antimicrobial		Topçu et al. (2017)
Anticholinesterase		Topçu et al. (2017); Şenol et al. (2010)
Cytotoxic		Topçu et al. (2017)
<i>S. ceratophylla</i> L.	Antioxidant activities	Topçu et al. (2017)
	Antibacterial activities	Bisio et al. (2019); Topçu et al. (2017)
<i>S. ekimiana</i> Celep & Doğan*	Antioxidant	Topçu et al. (2017)
	Anticholinesterase	Topçu et al. (2017)
	Enzyme Inhibitory	Topçu et al. (2017)

The antioxidant effect in such plants is generally related to the presence of phenols and flavonoids and their free radical scavenging activity (Baydar, 2013). The antioxidant activities of *Salvia* taxa are due to the main phenolic compounds such as carsonic acid, carnosol and rosmarinic acid (Cuvelier et al., 1996). Similarly, Poraz et al. (2017) stated that methanol and ethanol extracts of *S. aethiopis* and *S. ceratophylla* contain phenolic acid and there is a linear relationship between antioxidant capacity and the amount of phenolic acid. Phenolic compounds are important compounds due to their effects on the quality characteristics of foodstuffs such as appearance, taste, and taste, which are important for consumers, and their positive effects on human health as natural antioxidants (Nizamlioğlu and Nas, 2010).

Both plant extracts and essential oils of *Salvia* taxa exhibit a wide range of biological activities due to the compounds they contain. Studies on the genus *Salvia* have shown that some species belonging to this genus have properties that can be used in the treatment of various diseases. In general, *Salvia* species are plants that have antibacterial, antifungal, antiviral, antiseptic, analgesic, antioxidant, antispasmodic, antidiabetic, anticancer, etc. biological effects (Topçu et al., 2017). Topçu and Kusman (2014) stated that none of the drugs currently used in the treatment of Alzheimer's disease, which starts with the weakening of mental skills and progresses over time, are not sufficient, and therefore studies are continuing on new potential drugs of both natural and synthetic origin. They stated that the extracts could be used in the treatment of Alzheimer's disease. Also, extracts from the above-ground

parts of *S. cryptantha* exhibited anti-tumor activity against breast cancer (Özer et al., 2013), *S. hypargeia* and *S. sclarea* had anti-tuberculosis (Arya, 2011), *S. verticillata* showed anti-diabetic effect (Eidi et al., 2011). On the AChE enzyme, CHCl<sub>3</sub> extracts of *S.cyanescens* and *S.candidissima* ssp. *occidentalis* exhibited high inhibitory activity, while CH<sub>2</sub>Cl<sub>2</sub> extracts of *S.cryptantha* showed moderate inhibitory activity (Orhan et al., 2007; Orhan et al.,2013). On the other hand, the petroleum ether extracts of *S. ceratophylla* and *S.cyanescens* strongly inhibited the BChE enzyme (Orhan et al., 2007). It has been reported that root extracts of *S. hypargeia* have cytotoxic activity, while *S. sclarea* exhibits cytotoxic activity on L6 cells (Topçu et al., 2009).

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## CHAPTER 12

### THE MINERAL MATTER CONTENTS OF SALVIA TAXA IN FLORA OF TÜRKİYE

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## **INTRODUCTION**

Medicinal and aromatic plants (MAPs) have been an integral part of daily life and culture all over the world for centuries. MAPs contain secondary metabolites such as alkaloids, glycosides, saponins, essential oils, bitter substances, and tannins in different parts such as roots, stems, leaves, fruits, seeds, and flowers that can treat various ailments in humans and animals. The collection and cultivation of MAPs have continued throughout history (Adhikari et al., 2010; Nohutcu et al., 2019). The production and consumption of MAPs and products used in pharmaceuticals, cosmetics, food, etc. obtained from these plants are constantly increasing in the world and in our country (Solomou et al., 2016; Lubbe and Verpoorte, 2011).

MAPs, which have a wide range of uses, are also widely used in the food industry. The fact that these plants show biological activities such as antioxidant and antimicrobial has been effective in the increase in the usage area in the food industry (Laranjo et al., 2019). In addition, the aromatic properties of these plants also have very important functions for the food industry. For food purposes, these plants are used fresh or dried. The leaves, flowers, fruits, seeds, roots, tubers, and all above-ground parts of plants are evaluated by crushing or grinding. The most common use of medicinal and aromatic plants is in the form of spices (Pop et al., 2019). However, these plants are used as herbal tea, food supplements, and additives. It is known that herbal teas were used in different parts of the world before drinks such as black tea and coffee, which are widely consumed today. It is known that herbal teas are consumed because of their pleasing flavors as well as their healing

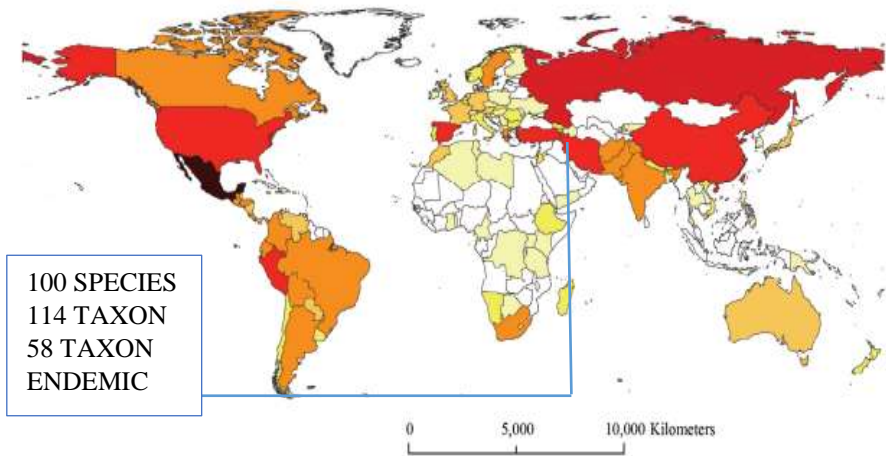
properties for some health problems. Herbal teas are preferred because of their positive effects on health rather than their nutritional properties (Ravikumar, 2014). Nowadays, herbal tea is produced from many plants such as *Salvia* spp., *Tilia* spp., *Mentha* spp., *Foeniculum vulgare* var. *dulce*, *Matricaria chamomilla*, *Echinacea* spp., *Rosa canina*, *Sideritis* spp., *Melissa officinalis*, *Rosmarinus officinalis*, *Artemisia dracunculus*, *Origanum/Thymus* spp., *Urtica* spp., *Ocimum basilicum*, and *Pimpinella anisum*. Another common usage area of medicinal and aromatic plants is food supplement products. In order for an herbal product to be considered a food supplement, it must contain essential nutrients such as vitamins, minerals, amino acids, and one or more chemical substances in the plant must be purified. Many plant species around the world are consumed in different ways as a source of natural healing. The most common form of consumption is herbal teas prepared with hot water. In this way, the chemical compounds in the plant pass into the water.

It is known that there are more than 10,000 medicinal and aromatic plants consumed as food in the world. Medicinal and aromatic plants used for food have an important place in food consumption, as they provide sufficient amounts of crude fiber, fat, carbohydrates, protein, water, and mineral elements such as Ca, Na, Fe, P, Mg, Zn, as well as vitamins (Hwiyang et al., 2010; Nagedra et al., 2008). In this context, *Salvia* taxa in the Lamiaceae family have great potential.

In this study, the mineral content of some *Salvia* taxa was evaluated with the data obtained from the studies we conducted and the current literature.

## 1. DISTRIBUTION AND USE OF SALVIA TAXA IN THE WORLD AND IN TÜRKİYE

The genus *Salvia*, a member of the Lamiaceae family, has a very rich species diversity throughout the world (Dweck, 2000). There are over 1000 *Salvia* species distributed worldwide. The worldwide distribution of *Salvia* species is presented in Figure 1 (Wei et al., 2015). There are 114 taxa of the genus *Salvia* in the Flora of Türkiye. 58 of these taxa are endemic. Türkiye is one of the important gene centers of *Salvia* species (Guner et al., 2012).



**Figure 1.** The worldwide distribution of *Salvia* species



The cultivar with the highest commercial value is *S.officinalis*. This species is not found in the flora of Türkiye, but it is successfully cultivated. *S.fruticosa* is also cultivated in Türkiye. However, *S.fruticosa*, *S.tomentosa*, *S.cryptantha*, *S.multicaulis*, *S. aramisis* and *S.sclarea* species are collected from the natural environment and presented to the domestic and foreign markets (Baydar, 2021).

Taxa in Lamiaceae family are rich in secondary metabolites. Therefore, they are important plants that can be used in various fields such as medicine, pharmacy, food, perfumery, and cosmetics. All aerial parts, leaves, and flowers of *Salvia* species are evaluated. These species are used in folk medicine as a sedative, pain reliever, cough suppressant, expectorant, and disinfectant in the treatment of various diseases and disorders. Some *Salvia* species are used as a spice. On the other hand, these species are consumed as herbal tea all over the world. Herbal teas are very popular today. The main reasons for this are the high number of medicinal plants and herbal tea varieties, low prices, side effects, and the harm they cause to the environment. In addition, recent support for the effects of drugs is an important factor (Pohl et al., 2016). Herbal teas contain various minerals (iron, magnesium, potassium, zinc, etc.) and vitamins. In addition, they have an important potential in terms of antioxidant compounds (Milani et al., 2019). For this reason, they are often used in alternative medicine.

## **2. THE MINERAL MATTER CONTENTS OF *SALVIA* TAXA IN FLORA OF TÜRKİYE**

*Salvia* species collected from the natural environment for different purposes can be harmful to health when used unconsciously or consumed more than necessary. It is known that these species are used very widely, especially as herbal tea (Gil et al., 2011; Rubio et al., 2012). Mineral elements have an important place in the life of living things. When they are more or less, they cause various problems in human life (Abacı Bayar, 2021).

Calcium (Ca), phosphorus (P), potassium (K), sulfur (S), sodium (Na), chlorine (Cl) and magnesium (Mg) are major minerals, while iron (Fe), cobalt (Co), copper (Cu), zinc (Zn), manganese (Mn), iodine (I), bromine (Br) and selenium (Se) are minor minerals in diets (Yadav et al., 2017). The studies on the mineral element content of *Salvia* taxa in the Flora of Turkey are summarized in Table 1 and 2.

According to the evaluated literature data, *Salvia* taxa have contained macrominerals Ca, Mg, and K in higher concentrations, and these three elements were followed by P. On the other hand, S was detected in fewer taxa (Table 1). High concentrations of microelements Fe, Mn, Zn, and Cu had determined in *Salvia* taxa. B was recorded in very few taxa (Table 2).

**Table 1.** The macrominerals concentrations of some *Salvia* taxa in Flora of Türkiye

Species	Macrominerals (mg kg <sup>-1</sup> )					References
	Ca	K	P	S	Mg	
<i>S. aethiopsis</i> L.	23500	900	-	-	6700	Abacı Bayar (2021)
	34900	1700	-	-	8400	
	200	400	40	10	-	Senkal et al. (2022)
	500	800	70	-	-	Doğan (2020)
<i>S. bracteata</i> Banks & Sol.	32600	1100	-	-	5600	Abacı Bayar (2021)
	55400	20	-	-	9000	
<i>S. ceratophylla</i> L.	34900	700	-	-	4100	Abacı Bayar (2021)
	28100	800	-	-	5100	
	38000	100	-	-	7200	
<i>S. absconditiflora</i> (Montbret & Aucher ex Benth.) Greuter & Burdet	16700	1300	-	-	3500	Abacı Bayar (2021)
	41700	500	-	-	7600	
	21300	400	-	-	4900	
	25946	12388	-	-	2158.7	
<i>S. cyanescens</i> Boiss. & Balansa	49300	200	-	-	8600	Abacı Bayar (2021)
	846800	135700	2376.7	-	-	Coşge Şenkal et al. (2019)
<i>S. dichroantha</i> Stapf	17562	21861	1910	2257	-	Coşge Şenkal et al. (2019)
<i>S. glutinosa</i> L.	16600	29700	4410	-	-	Coşge Şenkal et al. (2019)
<i>S. halophila</i> Hedge	12402	24171	1481	1483	-	Coşge Şenkal et al. (2019)
<i>S. heldreichiana</i> Boiss. ex Benth.	12855	15311	1873	1625	-	Coşge Şenkal et al. (2019)
<i>S. hypargeia</i> Fisch. & C.A.Mey.	69684	1636.5	-	-	3780.1	Gezek et al. (2019)
<i>S. nemorosa</i> L.	15700	15800	2480	-	-	Coşge Şenkal et al. (2019)
	16.52	10.70	910	-	-	
	23.56	-	-	-	-	
<i>S. officinalis</i> L.	1656	810	92	-	-	Coşge Şenkal et al. (2019)
	11131	11568	672	1714	-	
	15400	19200	1500	-	-	
	14582	23582	1750	-	-	
<i>S. sclarea</i> L.	4089.1	28021	-	-	3005.2	Gezek et al. (2019)
	455	738	101	-	-	Doğan (2020)
	12900	37500	2150	-	-	Coşge Şenkal et al. (2019)

**Table 1.** (Continued)

Species	Macrominerals (mg kg <sup>-1</sup> )					References
	Ca	K	P	S	Mg	
<i>S. syriaca</i> L.	23500	1200	-	-	3300	Abacı Bayar (2021)
	46300	800	-	-	8600	
	19000	1200	-	-	2700	
	16700	1300	-	-	3800	
<i>S. tomentosa</i>	18553	14518	1385	1034	-	Coşge Şenkal et al. (2019)
	11200	4000	900	-	-	
<i>S. verticillata</i> L.	-	5900	1400	-	-	Coşge Şenkal et al. (2019)
	191	915	77	-	-	
<i>S. virgata</i> Jacq.	50800	1700	-	-	6000	Abacı Bayar (2021) Gezek et al. (2019) Coşge Şenkal et al. (2019)
	18142	24566	-	-	5293.1	
	102.825	267.273	33.028	14.28	-	
	22400	7900	800	-	-	
<i>S. viridis</i> L.	22400	7900	800	-	-	Coşge Şenkal et al. (2019)

Mineral substances constitute 4-6% of the human body. They are very important in terms of nutrition. Both deficiency and excess of these in our body cause serious health problems (Kızıl et al.2010). Micronutrient deficiency affects more than two million people worldwide, resulting in worsening health and increased mortality (Pankaj et al., 2013). According to the World Health Organization, Fe deficiency is the most common malnutrition in the world (Arollado et al., 2010). *Salvia* species are mostly consumed as tea and spice. It is known that many *Salvia* species are rich in mineral substances. Therefore, *Salvia* taxa can make significant contributions to meeting the daily mineral needs of the human body.

**Table 2.** The microminerals concentrations of some *Salvia* taxa in Flora of Türkiye

Species	Micro minerals (mg kg <sup>-1</sup> )					References
	Fe	Mn	Zn	Cu	B	
<i>S. aethiopsis</i> L.	525	67	41.94	6.01	-	Abacı Bayar (2021)
	687	19.50	31.64	5.60	-	
	5.31	0.78	1.09	0.21	0.59	Senkal et al. (2022)
	12.99	0.59	0.99	0.44	0.82	Doğan (2020)
<i>S. bracteata</i> Banks & Sol.	787	40.00	31.99	5.34	-	Abacı Bayar (2021)
	3479	61.60	28.39	7.46	-	
<i>S. ceratophylla</i> L.	253	40.25	21.50	6.78	-	Abacı Bayar (2021)
	317	8.25	30.99	6.38	-	
	1149	50.05	45.05	7.67	-	
<i>S. absconditiflora</i> (Montbret & Aucher ex Benth.) Greuter & Burdet	366	75.50	32.19	6.50	-	Abacı Bayar (2021)
	347	29.75	28.69	6.99	-	
	729	18.75	37.98	5.51	-	
	524	19.30	39.78	4.78	-	
	479.7	46.14	31.16	5.876	-	Gezek ve ark. (2019)
<i>S. cyanescens</i> Boiss. & Balansa	626	77.00	28.07	6.89	-	Bayar (2021)
	108.48	12.36	33.27	82.4	-	Coşge Şenkal et al. (2019)
<i>S. dichroantha</i> Stapf	442	38.89	21.91	6.86	45.31	Coşge Şenkal et al. (2019)
<i>S. glutinosa</i> L.	508.26	49.47	93.75	59.75	-	Coşge Şenkal et al. (2019)
<i>S. halophila</i> Hedge	214	15.72	22	6.15	36.58	Coşge Şenkal et al. (2019)
<i>S. heldreichiana</i> Boiss. ex Benth.	179	29.79	23.53	4.99	32.71	Coşge Şenkal et al. (2019)
<i>S. hypargeia</i> Fisch. & C.A.Mey.	1609	69.06	24.33	8.332	-	Gezek ve ark. (2019)
<i>S. nemorosa</i> L.	167.62	46.26	34.25	36.70	-	Coşge Şenkal et al. (2019)
	280	-	50	-	-	
	379.27	-	35.02	-	-	
<i>S. officinalis</i> L.	297.4	32.6	48.4	35.8	-	Coşge Şenkal et al. (2019)
	44	3	6	1	-	
	565	38.8	28.7	4.67	37.8	
	453.77	39.25	64.46	25.10	-	
<i>S. reflexa</i>	350.54	46.53	18.84	34.18	-	Coşge Şenkal et al. (2019)
	189.5	9.46	18.32	128.2	16.3	Coşge Şenkal et al. (2019)
<i>S. sclarea</i> L.	1201	102.7	33.71	11.89	-	Gezek et al. (2019)
	8.14	0.93	0.99	0.30	0.81	Doğan (2020)
	366	68.75	37.52	7.30	-	
<i>S. syriaca</i> L.	1357	97.00	31.55	5.95	-	Abacı Bayar (2021)
	243	4.75	28.65	7.77	-	
	641	29.25	25.35	5.59	-	
<i>S. tomentosa</i>	782	42.42	30.78	4.39	27.97	Coşge Şenkal et al. (2019)
<i>S. verticillata</i> L.	6.55	0.59	0.99	0.44	0.82	Doğan (2020)
	670	43.75	38.90	6.24	-	Abacı Bayar (2021)
<i>S. virgata</i> Jacq.	193.6	25.02	8.921	-	-	Coşge Şenkal et al. (2019)
	2.171	0.650	0.466	0.165	0.603	

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## CHAPTER 13

### DORMANCY AND GERMINATION OF *SALVIA* SPECIES: AN OVERVIEW

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## INTRODUCTION

*Salvia*, the largest genus of the Lamiaceae (Labiatae) family, represents an enormous and cosmopolitan community of about 1,000 species with a remarkable diversity. When *Salvia* species are examined according to the world, about 36 species in Europe (Hedge, 1972; Harley et al., 2004), about 500 species in Central and South America (Walker and Sytsma, 2007), about 200 species in West Asia, 90 species in central Asia/ Mediterranean and 100 species in East Asia (Walker et al., 2004) and 72 species in Africa (Santos and Ferna'ndez, 1986; Van Jaarsveld, 1999) have been reported in the sources.

This genus has different centers of diversity, one of which is in the Andes of South America, with 28 *Salvia* species recognized in Bolivia, most of which are endemic and native to the Andes (Wood, 2007). Other, Türkiye, as a country, is one of the important gene centers for the genus *Salvia*. In the flora of Türkiye, the genus *Salvia* is represented by 100 species and 107 taxa, and approximately 50% of these species are endemic (Tursun et al., 2021).

The genus *Salvia*, which has been used as a medicinal plant in many parts of the world since ancient times, is described in the book "Flora of Turkey and the East Aegean Islands" as follows; Herbaceous, semi-shrub or shrub, perennial, rarely biennial or annual, strongly aromatic plants. Stems of this genus erect or creeping, usually covered with glandular hairs or glabrous. The leaves are whole, lyrate or pinnatisect. The inflorescences are variously combined chymoses. Vertisillatrum (1-)2-10(-40)-flowered, often or spaced far apart. Calyx

bell-shaped, funnel-shaped or tubular with double lips; upper lip 3-toothed, not fully developed or slightly indented; lower lip with 2 teeth; The calyx in the fruit is more or less enlarged in the fruit and has a membranous structure. Corollae are white, yellow, pink, blue or violet, double-lipped, upper lip straight to orifice; lower lip has three lobes which broad concave middle lobe and 2 small lateral lobes; the tube is straight or curved, bent inward or bulging, with or without rings, with or without scales. Stamens are 2, a fertile theca with a short filament at the upper end and a short or very long connective bed, and a smaller fertile or subfertile theca (Stamen type A) or various forms of unproductive tissue at the lower end (Stamen type B); stamens normally articulated at the junction of filament and connective, not rarely (Stamen Type C). The staminodiums (the posterior pair of stamens) are always present and small. The stylus is 2-lobed. Nutlets are glabrous, oval, triangular or slightly circular, producing mucilage when wetted (Hedge, 1982). General view of *Salvia* L. plant is given in Figure 1.



**Figure 1.** General view of *Salvia* L. (Anonymous, 2016)

Although leaves, shoot tips, flowers and partly stems of *Salvia* species, which are important medicinal plants, are used, the most utilized and economically important part is the leaves. *Salvia* species have a very high market potential as they appeal to a wide consumer group (food industry, pharmaceutical, aromatherapy and chemical industry, landscape, herbalists selling as retail products) due to their characteristics.

Generally, *Salvia* species are consumed as herbal tea, although they are used as spice and sweetener, as well as in perfumery, cosmetics (Delamare et al., 2007) and folk medicine to treat microbial infections, cancer, malaria, inflammation and disinfect homes after illness, especially in the treatment of the most common diseases such as stomachache, cold and sore throat (Kamatou et al., 2008).

The genus name "*Salvia*" derives from the Latin word "Salveo" meaning "to save, to heal" (Karatoprak et al., 2020). *Salvia* (sage) species, important medicinal plants, have been prepared by tea, ointment, tincture or extract in traditional folk medicine since ancient times, used in the treatment of various diseases such as an analgesic, expectorant, carminative, sedative, antiperspirant, externally wound healing, cold, bronchitis, tuberculosis, menstrual disorders and stomachache. In the pharmacopoeias of many countries in the world, Sage species against sweating and fever; as a gas remover; a spasmolytic; antiseptic/bactericidal; astringent; mouth, tongue and throat mouthwash against inflammation; a wound healing agent; in skin and hair care; and it has been used as a medicine against rheumatism

(Kintzios, 2000). On the other hand, it has been reported that *Salvia* species are used for memory enhancement in European folk medicine (Perry et al., 2003). In Middle Eastern countries such as Jordan, Lebanon, and Syria, *Salvia* species have been reported to be prescribed as boiled tea to relieve abdominal pain, headache, stomachache, and to treat cancer, microbial infections, asthma, cough, and other pulmonary and urinary diseases (Cardile et al., 2009). In addition, the species is used as a traditional medicine all over the world due to its antitumor, antidiabetic, antioxidant and antibacterial properties (Topcu, 2006; Pop et al., 2016).

This therapeutic feature of *Salvia* species can be attributed to the important secondary metabolites in the plant. *Salvia* species contain phenolics and terpenoids as their major phytochemicals, especially diterpenoids, essential oils, sterols, flavonoids, sesquiterpenoids (Lu and Yeap Foo, 2002; Jassbi et al., 2012).

Essential oils and extracts of *Salvia* species have some important activities such as antimicrobial, antioxidant, anticholinesterase, improving cognitive performance and mood, reducing work-related stress, antimutagenic, anticancer, and anti-inflammatory (Jassbi et al., 2016). The pharmacological effects of *Salvia* essential oils are based on the presence of more than 100 active compounds, which can be categorized as monoterpene hydrocarbons, oxygenated monoterpenes, sesquiterpene hydrocarbons, diterpenes, isoprenoid compounds, and oxygenated sesquiterpenes (Baytop, 1986; Jassbi et al., 2016). Essential oils have been used in perfume making and flowers as blush (Baytop,

1999). Rosmarinic acid derivatives and flavonoids in their extracts are the most extensively studied compounds for this important biological effect (Orhan et al., 2013; Loizzo et al., 2014; Jassbi et al., 2016).

In addition, *Salvia* species are used as ornamental plants due to their beautiful appearance and fragrant. For examples, *Salvia splendens* Sellow ex Schult., which has a beautiful appearance with its red flowers, and *Salvia sclarea* L., which is known as musk sage due to its beautiful scent with large pink or white flowers. They are the most commonly used *Salvia* species for this purpose (Nakiboğlu, 1993). Another, *Salvia wiedemannii* Boiss, because of its high aesthetic and decorative value, fragrant and ground cover feature, can be used as an ornamental plant in park and garden arrangements (Yücel and Altınöz, 2001).

Among the *Salvia* species, *S. officinalis* L. (Dalmatian sage), the most well-known species, which has been accepted as an official drug in the British Pharmacopoeia, has an important place in international trade (Figure 2) (Topçu, 2006).



**Figure 2.** The photograph of *Salvia officinalis* L. (Dalmatian sage)

*S. officinalis* naturally spreads in the southern and central parts of Europe and the Western Balkans, especially in Dalmatia and Macedonia (Güner et al., 2012), and is cultivated in many countries such as Germany, Southern France, Hungary, Russia, Türkiye and America for trade.

Other *Salvia* species with the highest commercial value are *S. fruticosa* Mill. (anatolian sage), *S. lavandulaefolia* Vahl. (spanish sage), *S. sclarea* L. (clary sage), and *S. pomifera* L. (apple sage) (Sage: The Genus *Salvia*, 2000; Elmas, 2021). In particular, *S. hispanica* L. (chia) is a sub-tropical crop which recently reinforced its commercial value due to the high proportion of omega-3 in their grains (Brandán et al., 2019; Julio et al., 2022). Other *Salvia* species cultivated are *S. tomentosa* Mill. (balsamic sage), *S. columbariae* Benth. (chia, chia sage, golden chia, or desert chia), *Salvia miltiorrhiza* Bunge (red sage, chinese sage, tan shen, or danshen) and etc. (Sui, 2019; Grimes et al., 2020; Liu et al., 2020; Elmas, 2021).

Among the *Salvia* species grown in Türkiye, *S. fruticosa* is the most collected and used both for domestic consumption and exported. However, *S. tomentosa* is intensively collected from nature (Baydar, 2016).

The use of medicinal and aromatic plants, which have been used as natural products for centuries, as raw materials in industries such as pharmacology, cosmetics and food, is increasing day by day in the world market. In order to bring into the economy the species that may have medicinal properties in the natural flora, and to produce high

efficiency and quality according to both producer and consumer demands, first of all, there is a need for improved varieties by breeding, and seed material conforming to standards (Bayram et al., 2010). Seed is the basis of agricultural production. Therefore, seed germination is an important element for growing a plant. Detailed knowledge of germination patterns is important not only for successful cultivation, but also for understanding the dynamics and tolerance of abiotic factors such as the growth of species in arid areas. Seed germination depends on internal dormancy (such as genotype and maturity) and external factors (such as temperature, salinity, light and humidity conditions) (Jafarinia and Yazdanbakhsh, 2016; Pająk et al., 2019).

In the present book section, we aimed to cover and discuss the available germination knowledge involving their response to germination practices of *Salvia* species as well as possible dormancy characteristics of their seeds. In this study, to collect the corresponding data, Scopus (date of access: 28 January 2022 and revisited on 10 April 2022), PubMed (date of access: 20 February 2022 and revisited on 15 April 2022), ISI-WOS (date of access: 05 February 2022 and revisited on 05 April 2022) were carefully studied. The keywords used for this research were *Salvia*, dormancy, types of seed dormancy, mucilage, germination, priming, hormone, chemicals, biological applications, and *in vitro* germination, in combination between *Salvia* and the rest of the mentioned keywords, one by one. The systematic research was also conducted considering all the accepted or unresolved names of *Salvia* species, as reported in [www.theplantlist.org](http://www.theplantlist.org), accessed on 18 April 2022



(The Plant List, 2022), alone or in combination with the previous terms, one by one.

## 2. DORMANCY IN SEEDS OF *SALVIA* SPECIES

Seed dormancy is the intricate adaptive feature of plants in which seed is incapable to germinate due to its integrity and vigor in a definite time period under whether specific/natural ideal growth condition or customized biotic factors viz., photoperiod (light/dark), temperature etc. that favors the non-dormant seed germination afterwards (Chen et al., 2022; Yan and Chen, 2020; Finch-Savage and Leubner-Metzger, 2006; Baskin and Baskin, 2004). When the 4-6 weeks old fresh seed do not germinate in spite of favorable condition, considered as dormant (Ensslin et al., 2018). Seed dormancy is ecological and physiological version that 1) heighten the plant survival 2) tolerates hostile environmental conditions 3) thwart seed from uniform germination 4) maintain seed vitality of wild plants 4) species continuation for evolution and (Stevens et al., 2020; Chen et al., 2022). According to Chen et al. (2022), 60-90% seeds of wild plants unveil dormancy. For the conservation point of view and fast retrieval of endangered plant species, dormancy become challenge for horticulture purposes due to the requirement of large amount of plant seedlings (Cho et al., 2018; Gao et al., 2021; Chen et al., 2022).

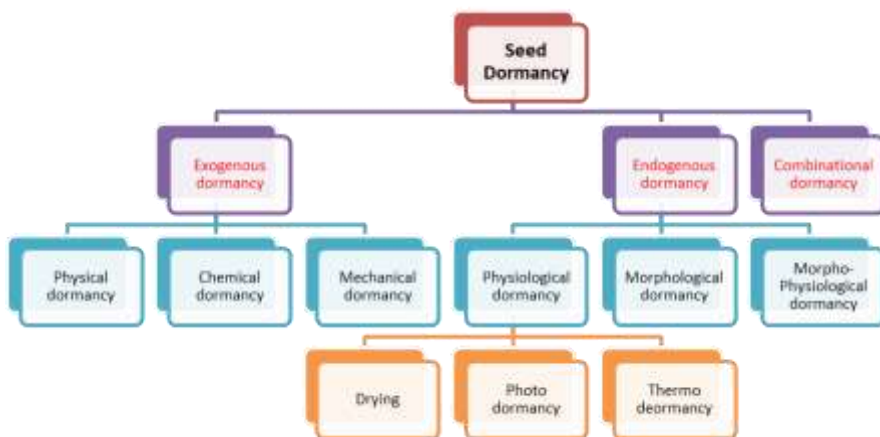
A matured dormant seed which develop from mother plant during seed maturation is called *primary dormancy* (Bewley, 1994; Hilhorst, 1995; Hilhorst et al., 1998) contrary to non-dormant seed that has the capacity to germinate under the diverse normal biotic and physical

environmental aspects like light, temperature, salinity etc. but it does not germinate due to the absence of above mentioned features, it is called pseudodormant (state of quiescence) (Hilhorst and Karssen, 1992; Koornneef and Karssen, 1994). In *S. aegyptiaca*, high salt concentration significantly decreases percentage and rate of germination pattern (Gorai et al., 2011).

Sometimes the plasma membrane of post dormant seeds lost their sensitivity of receptors after dispersal on the onset of unfavorable conditions, they are called *secondary dormancy*. Bewley and Black, (1985) explain that some environmental factors like absence of light prevent the seed to germinate but some quiescent non-dormant seeds require substrate moisture. Absolute light is required for the germination of *Salvia aegyptiaca* seeds (Sen and Chatterji, 1968). Light enhance the germination of *S. pomifera* whilst red/white light inhibited the germination of *S. fruticosa* (Thanos and Doussi, 1995).

### **2.1. Types of Dormancy**

There are three main types of seed dormancy *viz.*, exogenous dormancy, endogenous dormancy and combinational dormancy (both exogenous and endogenous). The Figure 3 shows all types of dormancy.



**Figure 3.** Types of seed dormancy

### 2.1.1. Exogenous dormancy

This type is mainly caused by external condition of seed's embryo that prevent germination due to the tough seed coat which robust to moisture penetration. Exogenous dormancy is further classified into three subgroups:

- Physical dormancy
- Chemical dormancy
- Mechanical dormancy

#### **Physical Dormancy**

In the seed coat /fruit coat, presence of 1-2 water resistant layers of palisade cells caused physical dormancy (Offord and Meagher, 2009; Baskin et al., 2000) so gases or water cannot be taken up by seeds. A special anatomical structure in seed (fruit) coat is formed called water gap (opening) to allow water to move to embryo can break such type of dormancy (Baskin et al., 2000). Whilst variation in temperature, freezing/thawing and fire can cause the natural breakdown of physical

dormancy. Moreover, disrupting the seed coat in region except strophiole (lens) by heating can also collapse the physical dormancy (Morrison et al., 1998). However, physical dormancy is assumed to be more advance than 100 million years ago (Baskin et al., 2000). Some *Salvia* species are affected by light-temperature cofactor. For example, *S. officinalis* is influenced by light and below 20 °C, its germination is inhibited but stimulate above 20 °C (Oberczian and Bernath,1988). However, *S. sclarea* germination was unconcerned to light (Oberczian and Bernath, 1988).

### **Chemical dormancy**

In seed coat, many growth regulators are accumulated around the embryo in coverings thus causing chemical dormancy. These are easily percolated by washing or soaking seeds in water. Many chemicals that avert germination are washed off from seeds by snow melts and rainwater. It is reported that 500 ppm of gibberalic acid treatment on *Salvia nemarosa* significantly increases the growth (Ahmadi et al., 2017).

### **Mechanical dormancy**

Sometimes embryo is unable to expand during germination due to the presence of very hard coverings and seed coat. Such type of dormancy is called mechanical dormancy. Mechanical dormancy has been reported in many species. Seed scarification can break mechanical dormancy by weakening the covering layer of embryo so that the radical can easily penetrate due to lower resistance. Detipped seeds have more negative (by c. 0.5 MPa) osmotic potential as compared to seed of

*Lycopersicon esculentum* because their testa layer and endosperm (opposite the radical) is removed by germinating in PEG (polyethylene glycol) solution (Groot and Karssen, 1992). Germination of *Salvia verbenaca* and *Salvia vernalis* decreases more promptly with reducing osmotic pressure. Germination of *S. verbenaca* and *S. vernalis* was 5% and 30%, respectively at -0.4 MPa osmotic potential and completely inhibited at osmotic potential of -0.6 MPa (Javaid et al., 2018).

### **2.1.2. Endogenous Dormancy**

Under development of embryos and the absence of specific seasonal signs lead to some chemical changes in seed cause endogenous dormancy. Sometimes endogenous chemical inhibitors can repress the germination. Endogenous dormancy is divided into three subgroups:

- Physiological dormancy
- Morphological dormancy
- Morpho-physiological dormancy

#### **Physiological dormancy**

This dormancy thwarts seed germination and embryo growth in anticipation of chemical changes. Physiological dormancy involve: 1). germination rate increases after the application of gibberellic acid (GA3) or after-ripening (dry storage) 2). Production of healthy seedlings after excision of dormant seed embryos 3). Increase germination due to warm (=15 °C) or cold (0-10 °C) stratification 4). Dry after-ripening shortens the cold stratification period required. By stopping the production of inhibitory chemicals using temperature fluctuation, physiological dormancy can be broken down. Abscisic acid

is a strong ‘growth inhibitor’ and light affects its production. Physiological dormancy is divided into three subgroups (Table 1).

*S. hispanica* seeds are heterogenous physiologically, comprising negatively photoblastic subpopulations at 35 °C and positively photoblastic subpopulations at 15 °C; though germination is light indifferent between 20-31 °C (Labouriau and Agudo, 1987). Physiological dormancy is divided into three subgroups.

**Table 1.** Illustration of three subgroups of physiological dormancy

Drying	Photo dormancy	Thermo dormancy
Some plants including those from seasonally arid regions need a period of drying before they will germinate; the seeds are released but need to have lower moisture content before germination can begin. If the seeds remain moist after dispersal, germination can be delayed for many months or even years. Many herbaceous plants from temperate climate zones have physiological dormancy that disappears with drying of the seeds.	Light sensitivity affects germination of some seeds. These photoblastic seeds need a period of darkness or light to germinate. In species with thin seed coats, light may be able to penetrate into the dormant embryo. The presence of light or the absence of light may trigger the germination process, inhibiting germination in some seeds buried too deeply or in others not buried in the soil.	Seed sensitivity to heat or cold. Some seeds germinate only at high temperatures (30°C or 86°F). Many plants that have seeds that germinate in early to mid-summer have thermo dormancy and germinate only when the soil temperature is warm. Other seeds need cool soils to germinate, while others like celery are inhibited when soil temperatures are too warm. Often thermo dormancy requirements disappear as the seed ages or dries.

### Morphological dormancy

In morphological dormancy, embryo is differentiated into hypocotyl–radicle and cotyledon(s). Some seeds contain non-physiologically dormant differentiated embryos that need more growth before radical protrusion. Furthermore, at the onset of fruit ripening, embryos are undifferentiated and seed ripe when they come in contact with water on ground. So the period between the fresh seed incubation

and radical emergence is dormancy period (Baskin and Baskin, 1998). Moreover, a special morphological feature ‘mucilage’ in *S. aegyptiaca* act as a sorbent or filter to prevent the seed from desiccation and harmful effects of salt (Yang et al., 2010).

### **Morpho-physiological dormancy**

It’s the combination of morphological and physiological dormancy. Seeds have immature embryo with a physiological constituent of dormancy. Thus a dormancy flouting pretreatment is required for seed germination. Embryo requires longer time for germination than usual.

#### **2.1.3. Combinational dormancy**

It comprises both the exogenous (impermeability of seed coat) and endogenous (Physiologically dormant embryo) dormancy (McDonald et al., 2005). Sometimes seed show conditional dormancy i.e., after maturity they germinate in field or in dry storage even while the seed coat remains impermeable to water (Baskin and Baskin, 1998). Some *Salvia* species require cold stratification for couple of weeks, after that physical dormancy is broken; water penetrates into seeds before germination.

### **2.2. Mucilage**

Mucilage is dense, sticky substance manufactured by fruit/seeds of plant species belonging to 110 families and 230 genera of angiosperms specially from Lamiaceae, Acanthaceae, Asteraceae, Boraginaceae, Cruciferae and Scrophulariaceae (Yang et al., 2010; Yang et al., 2012; Baskin and Baskin, 2014; Gorai et al., 2014; Zhang

et al., 2020). Mucilage is predominant form of celluloses, hemicelluloses, minerals, lipids, pectins with less amount of protein and it is chemically resembles to materials of primary cell wall (Ahmed et al., 2014; Cui, 2019; Nazari et al., 2020) and high molecular weight compounds produced by Golgi apparatus of seeds/fruits epidermal cells (Western et al., 2000; Gorai et al., 2014). Furthermore, mucilage is highly adaptive character for plants to survive in extreme dry (desert) conditions (Gorai et al., 2014). Mucilage is hydrophobic due to presence of acidic potential and polysaccharides and it protect the seed with jelly like layer after imbibes water. Along with suberin, and pectin, mucilage help in impermeability of seeds. Moreover, 30% seed mass is comprised of hydrophilic mucilage which has the ability to attain 550% in dry seeds (Withers and Cooper, 2008; Gorai et al., 2014).

In mucilage mass, pectin is considered as chief constituent but hemicelluloses are rich in seed envelope (Naran et al., 2008; Saeedi et al., 2010; Phan et al., 2016). In addition, cellulose fibrils attached to seeds and making network with pectins and hemicelluloses forming ‘cellulosic mucilage’ that is present in *Salvia* spp. and *Ocimum* spp. (Naran et al., 2008; Kreitschitz et al., 2021). In angiosperms, many organs like stem, leaves, seeds, roots and fruits deliberating a remarkable diversity of physical properties by producing mucilage (Galloway et al., 2020). In Lamiaceae (subfamily Nepetoideae), 60% fruits related to 400 species produce mucilage so mucilage production is clade (group) oriented. Much *Salvia* spp. produce mucilage from its outmost cell layer of fruits, termed non-dehiscent fruit pericarp



(achene) instead of seed integument (Ryding, 2001; Yang et al., 2012; Viudes, et al., 2020).

Ecological and physiological role of seed mucilage is mystery but its production is a big metabolic investment. Figure 4 depicts function of seed mucilage of *Salvia* spp.



Figure 4. Functions of Seed Mucilage in *Salvia* spp.

### 2.2.1. Physiological and physiochemical effects of mucilage on dormancy

*Salvia hispanica* also called ‘chia’ seeds imbibe water and release mucilage which is polysaccharide in nature. Chia mucilage is mainly composed of proteins, ash, fats, uronic acid (galacturonic acids and glucuronic acid) and carbohydrates including glucose, mannose,

galactose, xylose and arabinose. Many physiochemical variations are pragmatic reliant on geographic source, variety of seed and mucilage removal method (Chiang et al., 2021). GSOs (galactosyl-sucrose oligosaccharide) are used to enhance the valuable bacterial growth in gastrointestinal track and are prebiotics, constitute the major part of mucilage (Xing et al., 2017). Chia mucilage-polyvinyl alcohol is helpful in encapsulation of green cardamomum essential oils causing antimicrobial potential (Bustamante et al., 2017; Dehghani et al., 2020). By applying electrohydrodynamic atomization, mucilage-sodium alginate particles are obtained with varying mechanical and morphological properties (Song et al., 2017; Renteria-Ortega et al., 2021). In addition, oil nanoparticles of Chia mucilage have been produced (de Campo et al., 2017).

Muñoz et al. (2012) characterized and produce biodegradable and eatable mucilage thin films, rich with proteins. Such films have antimicrobial and nontoxic activities and play a key role in pharmaceutical productions. Hence in powder form, they are the best disintegrant and aided in dissolving tablets (Mujtaba et al., 2019; Madaan et al., 2020). Several methods have been adopted to extract chia mucilage from seeds viz., freeze-drying, oven-drying, agitation ultrasonic assisted and seed-solvent contact times using different parameters which affect the properties and extraction yield (Velázquez-Gutiérrez et al., 2015).

*Salvia macrosiphon* Boiss. also called wild sage is medicinal plant has multifunctional activities. It is biologically sound and

chemically rich species of *Salvia*. Its mucilage is used in packing and food items (low glycemic index) (Naji-Tabasi et al., 2021). It has prebiotics effect, so directly increase the resistance of gastrointestinal probiotic bacteria (Nasiri et al., 2021). Mucilage can be used as thin film as packing material by producing nanoclay with improved thermal, physical and mechanical features (Davoodi et al., 2020). These films have antimicrobial potential and biodegradable.

### **3. GERMINATION APPLICATIONS IN SALVIA SPECIES**

Most of the seeds of medicinal plants are variable in their ecological compatibility with environmental conditions. Therefore, it is necessary to create optimal conditions for seed germination of medicinal plants and to identify the eco-physiological factors affecting dormancy for their culture and production (Khakpoor et al., 2015). It is important for sowing and reproduction to provide information about the quality characteristics of seeds and to create the most suitable conditions for seed germination (Ghasemi Pirbaloti et al., 2007). The mucilage in the coats of the seeds of the genus *Salvia* is one of the biggest dormancy factors (Yaman, 2020). Due to the low germination rate of *Salvia* seeds, it is vital to examine the factors affecting the dormancy and germination of species belonging to this genus (Khakpoor et al., 2015; Yaman, 2020).

The *Salvia* species have been studied for their germination potentials that regard both priming, hormone treatments, wetting-drying with water applications, low-high temperature applications, liquid planting applications, some chemical substances, combination

applications and other applications affecting seedling emergence (Table 2).

**Table 2.** The germination rates of some *Salvia* species

<i>Salvia</i> species	Treatment type	The most chemical type	Most effective dose	Germination rate (%)	Reference
<i>S. columbariae</i> Benth.	Hormonal priming	GA <sub>3</sub>	200 mg/l GA <sub>3</sub>	>93	Hashemi and Estilai (1994)
<i>S. hispanica</i> L.			0.4 mM/L	85-100	Costa et al. (2021)
<i>S. fruticosa</i> Mill.			14 day+ 400 ppm GA <sub>3</sub>	33.3	Beken and Abdulhabip (2021)
<i>S. officinalis</i> L.			500 mg/l	About 100	Abdani Nasiri et al. (2018)
<i>S. officinalis</i> L.		Ascorbic acid	Ascorbic acid	71	Rezaie et al. (2013)
		GA <sub>3</sub>			
	Salicylic acid				
<i>S. aegyptiaca</i> L.	Hydropriming	Temperature	30 °C	>75	Gorai et al. (2011)
<i>S. hispanica</i> L.			20 °C	89.6	Cabrera-Santos et al. (2021)
<i>S. hispanica</i> L.			25 °C	92	Nadtochii et al. (2019)
<i>S. lerifolia</i> Benth			15 °C	92.8	Dashti et al. (2015)
<i>S. officinalis</i> L.			Temperature 30°, 12 h	85.5	Dastanpoor et al. (2013)
<i>S. splendens</i> Sellow ex Schult.			2 day at 20 °C	86	Jeong et al. (2000)
<i>S. aegyptiaca</i> L.	Osmopriming	NaCl	All dose	inhibitory	Gorai et al. (2011)
<i>S. candidissima</i> Vahl.		KNO <sub>3</sub> doses+ Pre-chilling	0 mg/L+ 7 day or 14 day	22	Şahin et al. (2011)
<i>S. cyanescens</i> Boiss. & Balansa		NaCl	1 % KNO <sub>3</sub>	66	Yücel (2000)
		KNO <sub>3</sub>			
<i>S. hispanica</i> L.		Salt treatment	10 mM NaCl	100	Younis et al. (2021)
<i>S. limbata</i> C.A.Mey		salinity treatments	50	53.8	Hashemi and Armaki (2015)
<i>S. nemorosa</i> L.		KNO <sub>3</sub> doses+ Pre-chilling	0 mg/L+ 7 day or 14 day	34	Şahin et al. (2011)
			2 mg/L+ 14 day		
<i>S. nemorosa</i> L.		NaCl doses	control	85	Kadioğlu (2021)
<i>S. officinalis</i> L.		NaCl	5 dS/m <sup>-1</sup>	68	Rezaie et al. (2013)
<i>S. officinalis</i> L.		NaCl doses	control	42	Kadioğlu (2021)
<i>S. spinosa</i> L.		NaCl	20 mM	About 95	Al-Turki et al. (2022)
<i>S. splendens</i> Sellow ex Schult.		PEG	-0.50 MPa and -0.75 MPa	82	Jeong et al. (2000)

**Table 2.** (Continued)

<i>Salvia</i> species	Treatment type	The most chemical type	Most effective dose	Germination rate (%)	Reference	
<i>S. splendens</i> Sellow ex Schult.	Osmopriming	KNO <sub>3</sub> , KH <sub>2</sub> PO <sub>4</sub> , K <sub>3</sub> PO <sub>4</sub> , NaOH, Ca(NO <sub>3</sub> ) <sub>2</sub>	50 mM and 100 mM KH <sub>2</sub> PO <sub>4</sub>	82	Jeong et al. (2000)	
<i>S. verticillata</i> L.		KNO <sub>3</sub> doses+ Pre-chilling	0 mg/L+ 7 day or 14 day	29	Şahin et al. (2011)	
<i>S. verticillata</i> L.		NaCl doses	control	80	Kadioğlu (2021)	
<i>S. verticillata</i> L.			50	79.4	Hashemi and Armaki (2015)	
<i>S. officinalis</i> L.	Biophysical priming	Magnetic field (M)	15 mT	About 60	Abdani Nasiri et al. (2018)	
<i>S. hispanica</i> L.	Light treatment		dark	91	Nadtochii et al. (2019)	
<i>S. spinosa</i> L.			light	About 90	Al-Turki et al. (2022)	
<i>S. cyanescens</i> Boiss. & Balansa	Acid treatment		All concentration in H <sub>2</sub> SO <sub>4</sub>	0	Yücel (2000)	
<i>S. siirtica</i> Kahraman, Celep & Doğan sp.			10 min in Citric acid of 4 %	9.8	Arslan et al. (2017)	
<i>S. siirtica</i> Kahraman, Celep & Doğan sp.	Stratification		28 day in fine sand maintaining in refrigerator of + 4 °C	21.25	Arslan et al. (2017)	
<i>S. officinalis</i> L.	Biological application	Plant growth promoting rhizobacteria (PGPRs)	<i>Pseudomonas putida</i> -41	78.5	Ghorbanpour and Hatami (2014)	
<i>S. officinalis</i> L.	Combination applications	Polimer+ Gibberellic acid	1000 ppm	88	Sönmez et al. (2019)	
<i>S. fruticosa</i> Mill.				85		
<i>S. hispanica</i> L.		Temperature, photoperiod	20 °C, light condition	88	Stefanello et al. (2015)	
<i>S. hispanica</i> L.				99	Paiva et al. (2016)	
<i>S. hispanica</i> L.		Saline water + in filter paper	0.42 dS m	94	Moghith (2021)	
				Saline water + in sand		96
<i>S. hispanica</i> L.		NaCl	KCl	-0.4 MPa	>70	Stefanello et al. (2019)
				-0.20 MPa	>80	
<i>S. miltiorrhiza</i> Bunge				150 mM CaCl <sub>2</sub> , 10 °C	50 >% >40	Gao and Yan (2020)
<i>S. sclarea</i> L.			Light and temperature treatment in paper	2 days open (room temperature) then 20 °C	89	Kumar and Sharma (2012)

**Table 2.** (Continued)

<i>Salvia</i> species	Treatment type	The most chemical type	Most effective dose	Germination rate (%)	Reference
		Light and temperature treatment in sand	room temperature open	76	
		Light and temperature treatment in pooled	3 days open (room temperature) then 20 °C	78	
<i>S. spinosa</i> L.		Temperature, 12 dark/12 light	15 °C	About 100	Al-Turki et al. (2022)
			10/20 °C	About 100	
			15/25 °C		
<i>S.verbenaca</i> var. <i>vernalis</i>		Temperature, photoperiod	30/20 °C (light/night), 12/12 h	>95.0	Javaid et al. (2018)
<i>S. verbenaca</i> var. <i>verbenaca</i>					
<i>S.verticillata</i> L.	Combination applications	GA <sub>3</sub> doses+ light application	2000 ppm GA <sub>3</sub> + dark	74	Tursun (2020)
		Sodium hypochlorite (0.5%)+ Application time+light application	Sodium hypochlorite (0.5%)+ 120 h+ light	55	
		Sodium hypochlorite (15%)+ Application time+light application	Sodium hypochlorite (15%)+ 60 min. + dark	51	
		Ethanol (96%)+ Application time+light application	Ethanol (96%)+ 60 min. + light/dark	28	
		Ethanol (3%)+ Application time+light application	Ethanol (3%)+ 120 h + light	47	
		Microwave 120 W (watts)+ Application time+light application	Microwave 120 W (watts)+ 20 h+ dark	58	
		Hydrochloric Acid (32%)+ Application time+light application	Hydrochloric Acid (32%)+ 15 min+ light and light/dark	46	

**Table 2.** (Continued)

<i>Salvia</i> species	Treatment type	The most chemical type	Most effective dose	Germination rate (%)	Reference
		Sulfuric acid + Application time+light application	Sulfuric acid + 120 sec + light/dark	68	
Other treatments					
<i>S. leriifolia</i> Bent.	Removing the seed coat			96.0	Estaji et al. (2012)
<i>S. macrosiphon</i> Boiss.	Single-wall carbon nanotubes (SWCNTs)		40 mg/L	59.6	Pourkhaloee et al. (2011)
<i>S. officinalis</i> L.	Nanoparticles	TiO <sub>2</sub>	60 mg/L, Nano TiO <sub>2</sub>	94.7	Feizi et al. (2013)
<i>S. miltiorrhiza</i> Bunge	Chemicals	La <sup>3+</sup>	30 mg/L	49.3	Sun et al. (2018)
<i>S. officinalis</i> L.	Magnetic pre-treatment		125 mT	65	Flórez et al. (2012)
<i>S. hispanica</i> L.	Sowing time		17 February	95	Goergen et al. (2018)
<i>S. officinalis</i> L.	Effect of sterilization		70% ethanol (5 min.) 5 % NaOCl (30 min.)		Досымбетова et al. (2021)
<i>S. splendens</i> Sellow ex Schult.	Storage conditions		5 °C, 24 months	84	Demir et al. (2020)
<i>S. officinalis</i> L.	Fertilizer treatments (vermicompost, Azotobacter, Azospirillum, cow manure)		(50% Vermi.Azos) 50% Azospirillum	14	Radnezhad et al. (2015)
<i>S. coccinea</i> Buc'hoz ex Etl.	Lead was used as nitrate solutions		100 mg/l	82	Stratu and Lobiuc (2015)
<i>S. candidissima</i> subsp. <i>occidentalis</i> Hedge	Heavy metal applications	CdCl <sub>2</sub>	10 ppm CdCl <sub>2</sub>	44	Gülçin (2021)
		PbCl <sub>2</sub>	100 ppm PbCl <sub>2</sub>	44	
		CuCl <sub>2</sub>	20 ppm CuCl <sub>2</sub>	44	
<i>S. coccinea</i> Buc'hoz ex Etl.		Zinc	100 mg/l, 144 h	>80	Stratu et al. (2014)
<i>S. multicaulis</i> Vahl.	Salinity in in vitro condition	KNO <sub>3</sub>	0.2 % KNO <sub>3</sub> , 24 h	23.3	Navabifar et al. (2013)
<i>S. stenophylla</i> Burch. ex Benth.		Light, smoke	Dark smoke	About 60	Musarurwa et al. (2010)
		Light, scarified			

	Different treatments in in vitro condition	Light, scarified-smoke			
		Light, control			

**Table 2.** (Continued)

<i>Salvia</i> species	Treatment type	The most chemical type	Most effective dose	Germination rate (%)	Reference
		Dark, smoke			
		Dark, scarified			
		Dark, scarified-smoke			
		Dark, control			
<i>S. cyanescens</i> Boiss. & Balansa	Different condition + 1 % GA <sub>3</sub>	Jacobson apparatus, room conditions, Rodewald apparatus, germination cabinet	Jacobsen apparatus, 1 % GA <sub>3</sub>	74	Yücel (2000)
<i>S. dorrii</i> ssp. <i>mearnsii</i>			Seeds under soil surface, 10 ml water/day	About 70	Kristin (1998)
<i>S. yosgadensis</i> Freyn & Bornm				100	Yaman (2020)
<i>S. virgata</i> Jacq.				40	
<i>S. ceratophylla</i> L.	In vitro germination		seed coat removal	100	
<i>S. sclarea</i> L.				100	
<i>S. candidissima</i> Vahl.				93.3	
<i>S. hispanica</i> L.	Moisture regim		4 ml for 100 seed in petri	92	Nadtochii et al. (2019)

#### 4. CONCLUSION AND RECOMMENDATIONS

*Salvia* is of great interest to food engineering, science and technology. It has huge antimicrobial, pharmaceutical and nutraceutical potential. Seed dormancy is a very adaptive trait in *Salvia* spp. It makes sure the population reproduction thus sinking the species competition by preventing the seed germination under unsuitable time. Sometimes dormancy become a curb to fortitude the seed quality and restricts the plant development and growth. Among other nutrients, the seed possesses mucilage composed mainly of carbohydrates and with



different functional properties and cast many physiological/physicomorphological effects on seed dormancy. Notwithstanding significant progress, methods of differentiation, secretion and synthesis of mucilage are under the weather and need to investigate the gene identification and function. Moreover, more detailed studies are required to compare a range of mucilage defects directly with each other under controlled conditions and in their ecological contexts.

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## CHAPTER 14

### MEDICINAL IMPORTANCE OF VIRGIN MARY THISTLE - MILK THISTLE (*Silybum marianum*)

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## INTRODUCTION

Since ancient times, human being has benefited from the plants around them to meet their needs such as food, fuel and medicine (Baytop, 1984). The medicinal plants, which started to be used in B.C. 3000 years, are called as an alternative medicine branch upon development of modern medicine in the 1900s. However, over the years, studies on medicinal plants and the substances obtained from those plants have increased the interest for these plants. Thus, researches on plants to find new natural pharmaceutical raw materials are increasing day by day (Baytop, 1984).

Virgin Mary Thistle (*Silybum marianum* (L.) Gaertner) is a naturally grown and cultivated annual plant belonging to the *Silybum* genus of the Asteraceae (Compositae) family (Eren & Şar, 2020). *Silybum marianum* seeds, which contain useful phytochemicals for liver diseases, are used safely and effectively in different regions of the world (Aktay et al., 2000).

*Silybum marianum* (= *Carduus marianus*) The Virgin Mary Thistle plant is an annual plant belonging to the Asteraceae (=Compositae) family, naturally grown and cultivated in our country. Its seeds have been used in the treatment of liver diseases for almost 2000 years, and its positive effect on liver diseases has been proven as a result of modern research and studies. This plant was given this name because it was seen as a religious symbol resembling the Virgin Mary in Germany. On the other hand, The Indians, called this plant Thistle, Sacred Thorn, Blessed Thorn. Research on the Virgin Mary Thistle

Plant started about 30 years ago in 1958. At the end of research for 10 years, a research group led by H. Wagner from the University of Munich succeeded in separating a compound known as silymarin from its seeds. (Sanchez-Sampedro et al., 2008).

The drug name of Virgin Mary Thorn and its naming in other languages are as follows;

Latin: *Silybum marianum*, *Carduus marianus*

It is known as “Meriendistelfüchte”, “Marienkörner” in German, “Chardon Marie” in French, “Carduo Mariano” in Italian and “Marietidsel” in Danish. Commonly known as “Marian thistle, wild artichoke, variegated thistle. It is also known as St. Mary's thistle, Christ's crown, holy thistle, Venus thistle, heal thistle, wand of God's grace". (Tenney, 1995; Baytop, 1999).

Turkish names: Deve diken, Akkız, Kenger otu, Akdiken, Mubarek Diken, Meryem Ana Diken, Yabani enginar, Deve kengeli, Sütülü kengel, Kıbbun, Şevkülmaryem.

The place of the plant in the systematic;

Section: Spermatophyta

Subsection: Angiospermae

Class: Dicotyledones

Team: Campanulales

Family: Asteraceae (Compositae)

Genus: *Silybum*

Kind: *Silybum marianum* L. Gaertner (Çelik, 2009)

There are 2 (two) subspecies of Virgin Mary Thorn in Türkiye. These are *S. marianum* ssp. *marianum* and *S. marianum* ssp. *anatolicum* dur. Both types contain the same active ingredients and the silymarin ratio is over 1%. *Silybum marianum* and *Carduus marianus* are mentioned as synonyms in DAVIS' Flora book (Tanker and Tanker, 2003). The plant is seen in South-Western Europe, Australia, North Africa and Western Asia. It is common in Mediterranean countries and south of Russia. It is also seen naturally in Cashmere, India-Pakistan Regions. It is also used as a vegetable in Mediterranean countries. It is seen in the Western and Southern Anatolia, in İzmir, Aydın, Denizli, Mersin, Adana and Antakya regions and in the coastal part of the Marmara region in Turkey; it grows on roads and field edges and in empty areas (WHO, 2002; Çelik, 2009).

### **1. MORPHOLOGICAL PROPERTIES**

*Silybum marianum* (*Carduus marianus*- Milk Thistle, Virgin Mary Thorn) is an annual herbaceous Mediterranean plant in 20-150 cm height, that is commonly found in empty fields, especially in our Aegean and Marmara coastal cities. The Virgin Mary thistle has a deep tap root, underdeveloped and a smaller number of lateral roots. The stem shows branching in the upper parts. The veins of the leaves are quite prominent and in white color. Its leaves are large pennatifid, bright green, with white veins and spiny lobes. The flower is a large capitulum. The flowers are hermaphrodite, red-pink, white or brown. The color of the seed varies from yellowish brown to black brown. 1000 grain weight of the seed varies between 22.1-31.2 g. seed; the seeds are dark-



colored oval average 6 mm long (Tanker et al., 2014; Çelik, 2009; Koç, 1997).



**Figure 1.** Purple flowered Virgin Mary's Thistle



**Figure 2.** White flowered Virgin Mary's Thistle



**Figure 3.** Virgin Mary Thistle



**Figure 4.** Ripe *S. marianum* seeds



**Figure 5.** *Silybum marianum* (L.) a view from the culture study of the gaertner plant-Konya (2005)

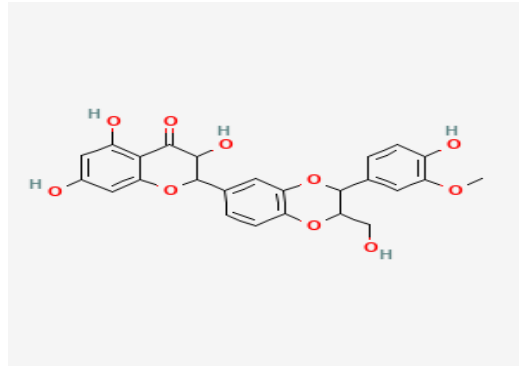
## 2. CHEMICAL COMPOSITION

The medicinally used part of the Virgin Mary's Thorn is its ripe seeds. Some active substances in its seeds are widely used, especially in liver diseases.

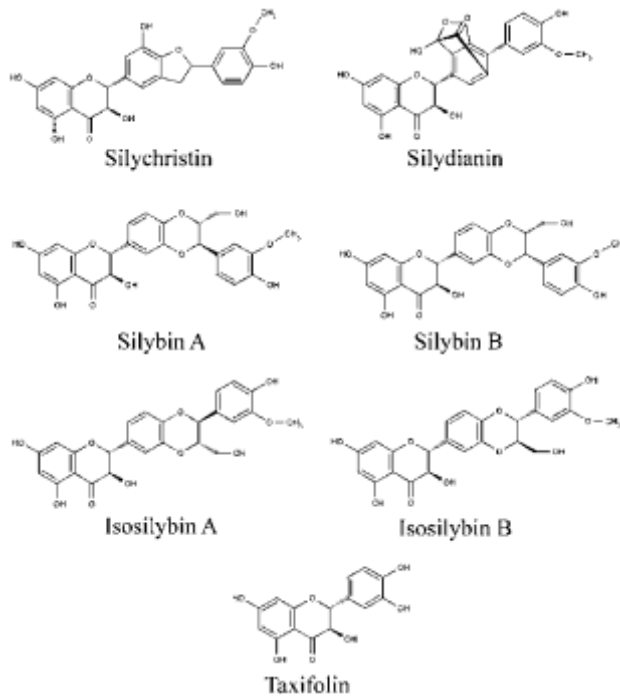
There are flavonoids, polyphenols, steroids and organic acids in Virgin Mary's Thorn herb. Flavonoids include apigenin-, luteolin- and kaempferol-7-O-glycosides, apigenin-4,7'-di-O-glucoside, kaempferol-7-O-glucoside-3-sulphate. Beta-sitosterol and beta-sitosterol glucosides are included in it. There is fumaric acid (3.3% on average) as organic acid in it.

There are apigenin, chrysoeriol, eriodictyol, naringenin, quercetin, taxifolin flavonoids in the seeds of Virgin Mary Thistle. In addition, it has 20-30% oil in the seed. The oil obtained from the seed is very rich regarding to linoleic. The most important component in its seed is silymarin (1,5-3%), which is a flavanolignan. *Silybum marianum* (milk thistle) containing silymarin complex includes silybin, silicristin, silidianin, dehydrosilybin, deoxysilikristine, silandrin, silibinom, silihermin, and neosilihermin. Silymarin has a complex structure and its main components are silybin A and silybin B, in other mean silybin. Other components are isosilybin A, isosilybin B, silychristin, silydianin. In this way, silymarin has a complex structure consisting of a mixture of multiple flavanolignan isomers. Silymarin is not found in other organs of Virgin Mary thistle such as leaves, stems and flowers. Silymarin is found only in the seed. Its chemical formula is  $C_{25}H_{22}O_{10}$ . One of the most important features of the silymarin

compound is its having a strong antioxidant effect. Its seed is known as Fructus Silybi mariani or Fructus Cardui mariae. (Gruenwald et al., 2004;- Kocaman and Dabak, 2015). The chemical structure of silymarin is given below;



**Figure 6.** Chemical structure of silymarin



**Figure 7.** Silymarin components and chemical structure of taxifolin

Another important component found in Mary Thistle Seed is taxifolin. Taxifolin is in a flavonoid structure and its other name is dihydroquercetin. Its chemical formula is  $C_{15}H_{12}O_7$ . The antioxidant effect of taxifolin is too high (Çelik, 2009).

### **3. USAGE AND MEDICAL IMPORTANCE OF MARY THISTLE**

*Silybum marianum* Gaertn (L.) has been known and used in European and Mediterranean countries since ancient times. When it is mixed with honey, the Roman naturalist, Plinus the Elder mentioned the effect of increasing bile flow, and he became the first reference to the use of this plant in liver diseases. Therefore, he gave the name Silybum to the plant. It was dedicated to the Virgin Mary in the early Christian period and was called Marian Thistle. The Virgin Mary thistle, which has been used for two thousand years, is mentioned in *Planta Medica* magazine with the expression "blessed plant from the past to the future". (Zeybek and Haksel, 2010; Ceylan, 1994). It has been known since the Middle Ages that its seeds have a specific positive effect on the liver (Çelik, 2009). Studies on the isolation of the active ingredient in the seeds of thistles began in 1958 and H. Wagner obtained silymarin in 1968. Since the active ingredient of the seed, silymarin, is not water-soluble, it cannot be consumed as herbal tea. The most common use type is standard encapsulated plant extract.

In Türkiye, 5% decoction prepared from dried powder or seeds mixed with honey is used in liver diseases and as a bile enhancer. Above-ground parts of the plant are used as diuretic, tonic, antipyretic,

rheumatic pain reliever and sedative; its fruits are used as regenerative for liver cells and increasing milk secretion (Baytop, 1999; Demirezer et al., 2007).

The drug is mostly used as a medicine with antihemorrhagic and hepatoprotective effects and has a widespread use in kidney and liver diseases. The above-ground part is used as diuretic, antipyretic, sedative, appetizing, and its fruits are used in rheumatic pains, liver diseases and bile increaser among the people (Tanker and Tanker, 2003; Baytop, 1999). It is used for the prevention and treatment of liver and bladder diseases, jaundice, hepatitis, fatty liver, chronic degenerative liver diseases, liver dysfunction after hepatitis, cirrhosis, and liver damage caused by metabolic toxins such as alcohol and tissue poisons for medical purposes. It is also used for poisoning caused by some chemicals and *Amanita phalloides* fungus and minimizing the side effects of chemotherapy. It is also preferred in allergic diseases due to its therapeutic properties in some sources. (Mat, 1997).

It is reported in the monographs of the World Health Organization (WHO) that silymarin and its components have antioxidant and antihepatotoxic effects in vitro, and that silybin also has an anti-inflammatory effect. In the same monograph; it is stated that standardized silymarin preparations within the scope of Clinical Pharmacology are used in the treatment of acute and chronic hepatitis caused by alcohol, organic compounds, drugs and toxins (WHO, 2002). It is well known that silymarin has antioxidative and chemo-protective properties, generally in the liver. The hepatoprotective activity of

silymarin is thought to be arisen from its antioxidant and membrane stabilizing properties. It also has a regenerative effect on the liver (Wu et al., 2009). At the same time, silymarin has started to take place in formulations in cosmetics in recent years due to its strong antioxidant properties. It prevents the entry of toxins into the cell and fights against liver damage through changing the structure of the outer membranes of liver cells. It helps to increase the production of bile, which plays a role in burning fat with its choleric effect, providing bile flowing. It is included in anti-aging and eye creams, body lotions and silymarin formulations with its seed oil (Yüce and Yener, 2012). It has been proven that silybin and silymarin are effective against two important hepatotoxins: amanitin and phalloidin, which are produced by the fungus *Amanita phalloides*. It has been reported that Silybin is highly effective against amanitin, especially when it is given with penicillin (Dixit et al., 2007). Silymarin is produced in the form of capsules or tablets in 250-750 mg doses as ethanol extracted. Although the amount of daily dose is different, it is recommended to take it 2-3 times in a day. Intravenous forms of Silybin are approved in Europe for *Amanita phalloides* mushroom poisoning. (Günşar, 2017).

In addition to the widespread use of silymarin preparations in the world as medicine and therapeutic, its oil obtained from its seed is also evaluated in the cosmetics field. It is used as a massage oil, also used in skin diseases (eczema, neurodermatitis, burns), vaginal inflammation, hemorrhoids, fissures, colitis and gastritis. Preparations obtained from its oil are offered to market as hand cream, sunscreen,

massage oil, and diaper rash cream. In some cases, like burns, erythema, melanogenesis, etc. on the skin exposed to the UV radiation of the sun; there are its preparations as creams. At the same time, Virgin Mary Thistle oil was bottled abroad and offered for direct consumption. Black cumin oil is consumed by people like centaury oil. It has internal use for therapeutic purposes, especially in cirrhosis, hepatitis and liver diseases, gastritis and colitis, stomach and duodenal ulcers. (Asaker-Abu, 2011; Çelik and Kan, 2013).

#### **4. CONCLUSION**

Virgin Mary Thorn, which has a wide range of uses in traditional medicine, is widely consumed today in the form of many pharmaceutical raw materials and preparations. Thanks to the silymarin contained in its seeds, silymarin, which has anti-inflammatory, anti-apoptotic, anticarcinogenic, antiviral, antifibrotic and antiangiogenic properties, is well tolerated and safe to use, is available to research in terms of its therapeutic aspects in different diseases therefore it's future looks bright, appears to be promising. Its herb is traditionally consumed in salads and meals. Preparations containing silymarin extract are sold in pharmacies and some markets in the world and in our country. Incentives can be made for cultivating the plant and expanding its agriculture in our country since Mary Thistle is mostly collected from nature and then consumed. More research and studies should be carried out to know its benefits for the liver through encouraging its consumption by more people.



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## CHAPTER 15

### NUTRITIONAL CONCENTRATION VALUES OF FIELD LARKSPUR (*Consolida regalis*) CONSUMED AS PURPLE MINT IN SIVAS

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## INTRODUCTION

The Ranunculaceae family contain 50 to 60 genera and 2,500 species. Some plants belonging to this family are highly poisonous and are used as herbal medicines or spices (Bruneton, 1999; Ipor and Oyen, 1999; Heywood et al., 2007; Kokoska et al., 2012). The *Consolida regalis* Gray is from family of Ranunculaceae (Bitiş et al., 2006). *Consolida regalis* grow on sandy or chalky soils at about 0–1,200 metres above sea level. It grows up on about 30–80 centimetres and the stem is erect. This plant is tolerant drought. The flowers of *Consolida regalis* consist with five to eight hermaphrodite flowers. the color of the flowers varies between dark blue and purple with five sepals (Anonymous, 2022). As a self-fertile plant, and the flowers which contain diterpenoid alkaloids (Anonymous, 2017). The alkaloids they contain of *Consolida* species cause them to be toxic. This toxicity can cause neurotoxic, muscle system spasms and even death (Meriçli et al., 2001; Gonz'alez et al., 2005; Bitiş et al., 2006). Thanks to the alkaloids it contains, *consolida* when used in appropriate amounts, have effects in analgesic balms, sedatives, emetics and anthelmintics (Meriçli et al., 2001; Ulubelen et al., 2001).

Plants rich in alkaloids are called alkaloid plants. Only 9% of the 10,000 plant species found in the world flora contain alkaloids. Although the plant is an alkaloid plant, the amount of alkaloids in its content is not very high. However, when taken in high doses, they can show poison, drug and addiction effects. industrially, alkalots are mostly used in the production of medicinal and agricultural drugs.

Alkaloids are very active pharmacologically because they contain nitrogen (Baydar, 2020).

We call the plants we use by drying and grinding to give taste, smell and color to the food products "spices", and the plants we obtain them "spice plants". At the same time, these plants have been used as a food preservative since ancient times. Spices contain on average 35-70% carbohydrates, 5-20% protein, 3-50% fat, 3-15% mineral substances and 8-14% water. In addition to K, Ca, P, Na, Fe and Zn, there are many valuable mineral substances in spice ash. Spice oils are very rich in essential fatty acids and give them their distinctive smell and taste. The nutritional energy values are generally between 250-500 kcal. Spices consumed as dry contain more vitamin C than those consumed fresh (Baydar, 2020). Spice plants that we use on our tables every day play a major role in food consumption and nutritional values.

In this study, the nutritional content of the *consolida* plant, which is consumed as purple mint in the vicinity of Sivas province, was determined.

## 1. MATERIAL AND METHODS

*Consolida regalis* was collected from the wild flora of Sivas Cumhuriyet University Campus. The determination of plant nutrient concentration values was carried out in The Laboratories of Sivas Vocational School, The Plant and Animal Production Department in the 2018 year. The collected plant samples were washed in the laboratory with tap water, 0.1 N HCl and then 2 times with distilled water. It was then placed in paper bags and dried in an oven at 70 °C for 48 hours.

Dry plant samples were ground into powder by grinding in an agate mill, and then 0.2 g was weighed, with a mixture of H<sub>2</sub>O<sub>2</sub>-HNO<sub>3</sub> acid (2 mL 35% H<sub>2</sub>O<sub>2</sub>, 5 mL 65% HNO<sub>3</sub>) in a microwave device (Milestone Ethos Easy Advanced Microwave Digestion System, Italy) for wet combustion. According to Murphy and Riley (1962), the phosphorus (P) concentration is colorimetrically at 882 nm in the spectrophotometer, calcium (Ca), magnesium (Mg), potassium (K), iron (Fe), manganese (Mn), zinc (Zn) and copper (Cu) concentrations were determined in Atomic Absorption Spectrophotometer device (Shimadzu AA 7000, Japanese), nitrogen concentrations were determined according to the Kjeldahl distillation method (Bremner, 1965). Each analysis was repeated three times in the study.

## **2. RESULT AND DISCUSSION**

The macro and micro nutrient concentrations of the plants collected from the university campus in the study are given in Table 1. In the study, nitrogen concentration of *Consolida regalis* plant from macro elements is 2.14% N, phosphorus concentration is 0.111% P, potassium concentration is 4.89% K, calcium concentration is 1.00% Ca, magnesium concentration is 0.57% Mg, iron concentration is 148.7 mg Fe kg<sup>-1</sup>, zinc concentration is 27.8 mg Zn kg<sup>-1</sup>, manganese concentration 15.6 mg Mn kg<sup>-1</sup> and copper concentration 9.1 mg Cu kg<sup>-1</sup> was determined (Table 1).



**Table 1.** Macro and micro element concentrations of *Consolida regalis*

Elements	Value
N, %	2.14 ± 0.06
P, %	0.111 ± 0.01
K, %	4.89 ± 0.08
Ca, %	1.00 ± 0.03
Mg, %	0.57 ± 0.01
Fe, mg kg <sup>-1</sup>	148.7 ± 5.36
Zn, mg kg <sup>-1</sup>	27.8 ± 2.37
Mn, mg kg <sup>-1</sup>	15.6 ± 1.54
Cu, mg kg <sup>-1</sup>	9.1 ± 1.21

When the data were evaluated as a whole, it was determined that the nutritional elements of the *Consolida regalis* plant were generally sufficient. In similar studies with medicinal and aromatic plants, it has been reported that the nutritional elements are generally at a sufficient level. Uçar et al. (2020) determined the nutrient elements in *Hypericum scabrum* and *Alchemilla mollis* plants collected from Sivas province. They determined nitrogen, phosphorus, potassium, magnesium, calcium, iron, zinc, manganese and copper concentrations of *Hypericum scabrum* plant 1.2% N, 0.214% P, 1.36% K, 0.85% Mg, 2.98% Ca, 458.9 mg Fe kg<sup>-1</sup>, 45.7 mg Zn kg<sup>-1</sup>, 187.2 mg Mn kg<sup>-1</sup>, and 21.7 mg Cu kg<sup>-1</sup> as respectively. Also they determined in *Alchemilla mollis* plant as 1.1% N, 0.036% P, 0.93% K, 0.78% Mg, 2.37% Ca, 511.2 mg Fe kg<sup>-1</sup>, 35.6 mg Zn kg<sup>-1</sup>, 255.8 mg Mn kg<sup>-1</sup>, 11.3 mg Cu kg<sup>-1</sup>. In another study, the nutrient concentrations of the *Arum maculatum* plant was collected from Kadirli, Düziçi (Osmaniye) and Andırın (Kahramanmaraş) regions were 3.46-4.79% N, 0.35-0.37% P, 2.67-2.82% K, 0.12-0.16% Mg, 0.04-0.06% Na, 0.49-0.68% Ca, 50.2-122.0 mg Fe kg<sup>-1</sup>, 38.7-62.3 mg Zn kg<sup>-1</sup>, 16.1-25.3 mg Mn kg<sup>-1</sup>, 0.0016-0.0023 mg Cu kg<sup>-1</sup> between were reported (Akpınar, 2021). In a study, Ucar et

al., (2021) examined the nutrient content of *Valeriana dioscoridis* Sm. plant, which they collected from eight regions of Antalya province. At the end of the research, they reported that nitrogen concentration of *Valeriana dioscoridis* Sm. plant 0.680-1.733 % N, phosphorus concentration 0.476-0.950 % P, potassium concentration 1.290-3.813 % K, magnesium concentration 0.493-0.880 % Mg, calcium concentration 0.083-8.803 % Ca, Mn concentration 63.97-1200 mg Mn kg<sup>-1</sup>, iron concentration 3126-4662 mg Fe kg<sup>-1</sup>, copper concentration 24.31-63.09 mg Cu kg<sup>-1</sup>, and zinc concentration 35.87-70.79 mg Zn kg<sup>-1</sup> varied between.

According to the report of the Baydar and Erdal (2004); the nutritional content of thyme plant are 2.9-3.6% K, 0.9-1.5% Ca, 1.0-1.2% N, 2024-2769 ppm Na, 1500-2400 ppm P. , 47.2-97.5 ppm Fe, 55.7-65.7 ppm Zn and 49.0-65.2 ppm Mn.

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