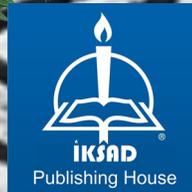


# AGRICULTURAL STUDIES ON DIFFERENT SUBJECTS

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Assoc. Prof. Dr. Arzu ÇIĞ



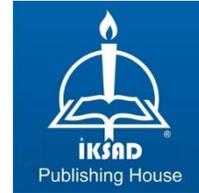
# AGRICULTURAL STUDIES ON DIFFERENT SUBJECTS

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## **PREFACE**

Every area where agriculture is done is valuable. Agriculture has the biggest role in taking precautions against drought, global warming, climate changes and environmental pollution caused by the increasing world population and globalization. The basic needs of nutrition and shelter are based on agriculture. Therefore, all kinds of researches in agriculture are very important in terms of revealing and applying knowledge and experience. I would like to thank all the authors who did not hesitate to share their experiences and knowledge with devotion in this book.

**Sincerely Yours**  
**Arzu ÇİĞ**



**CHAPTER I**  
**AGRICULTURAL ETHICS**  
**MULTIFUNCTIONAL AGRICULTURE?**

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## **INTRODUCTION**

Agriculture consists of all plant-livestock production activities that have been carried out for thousands of years for the most basic need of human beings -nutrition- and fiber production as a clothing raw material. With these features, agriculture is an indispensable and superior sector for the whole world.

Here it may be asked the unique value and purpose of agriculture is to grow things for human use, even though it is so obvious and simple, whether it is necessary to complicate it with ethical considerations (Dundon, 2003). In the past, the function of agriculture was seen only as feeding people. The concept of “multifunctional agriculture” has emerged as a key concept in scientific and political discussions on the future of agriculture and rural development (Renting et al., 2009).

The rapidly increasing World population, the problem of hunger, the living conditions of people working and living in agriculture, the awareness of healthy and safe food, the fact that the scarce resources of our aging world are running out; have led to the questioning of the techniques used and the mistakes made while carrying out agricultural activities and increasing production.

Essentially, agriculture with its feature of serving human nutrition, is not just a production activity, but a sector that has more than one function, including social welfare by its nature. From this point of view, it is clear that agriculture needs ethical values and rules. Of course, an

activity that includes humans and living things in the most general sense, done by humans and for the service of humans, must be carried on within an ethical framework. Agriculture is not only technical, because it is an economic, ecological and even strategically important branch of activity for the countries, apart from the nutrition of humanity, which concerns people and even all other living things and affects positively or negatively; are intertwined with ethical dilemmas and issues.

Ethics is one of the sub-disciplines of philosophy. Many things can be said about philosophy, but it is very difficult to make a general definition. Philosophy is a discipline that not only tries to understand the world, life and society, but also tries to make it meaningful and explain it. Philosophy questions and criticizes existing views, unquestioningly accepted ideas, pre-acceptances of sciences (Cevizci, 2018). Undoubtedly, this aspect of philosophy is a natural consequence of the fact that human beings, who are the subjects, have reason and willpower, unlike other living things.

Ethics, as a sub-branch of philosophy that deals with moral values; investigates the principles and foundations of morality and focuses on the requirements of being moral (Cevizci, 2018). Morality specifies the rules expected to be followed and the duties to be performed in interpersonal relations (Aktan, 2011). In other words, what is right or wrong in human behavior, actions and interpersonal relations; it can also be expressed as a set of norms that determine what is good or bad.

Ethical analysis involves thinking about and analyzing what people do, and tries to provide justifications for why their actions are right, based on moral theory (Zimdahl, 2006). Moral and ethical concepts emerged as a result of human needs and transformed in to different structures in line with changing human needs (Özdemir, 2020). As agriculture is an applied profession, agricultural ethics is a type of applied ethics just like many other professions.

Applied ethics is a trend that emerged after the 1970s –with the effect of technological developments- and started to become widespread. It is not completely independent of normative ethics and can be understood as the application of normative ethical theories in concrete situations that are controversial or cause dilemmas. Ethical problems encountered in different Professional activities are considered within the framework of applied ethics and principles and attitudes related to these problems are defined (Bayram, 2006). Agricultural and food ethics is one of the most comprehensive areas of bioethics, which is an applied ethics branch (Turhan et al., 2017). The concept of agricultural ethics can be defined as the whole of moral values and ethical principles that must be followed while carrying out agricultural activities. The reason for the formation of ethical dilemmas is that not all moral values, rules or principles are universal. Moral rules can vary from individual to individual, from society to society, and even according to time periods. An action or phenomenon that was previously accepted as morally and ethically right and good may be perceived as wrong or bad over time.

Environmental ethics emerged in the early 1970s as a new philosophical sub-discipline in the contemporary sense, although issues related to nature occupied a considerable place in 19th and 20th century philosophy (Akalin, 2019). Agricultural and food ethics which emerged later; It is a fairly young discipline of applied ethics, affected and nurtured by bioethics and environmental ethics. Paul B. Thompson (2017), who is considered the pioneer of agricultural ethics, described agricultural ethics as a sub-branch of environmental ethics in his book *The Spirit of the Soil*, published in 1995 (Thompson, 2017). Agriculture and food ethics emerged as a result of increasing concerns in the Western world with the changes in agriculture and food systems about thirty years ago. Although there are similar concerns in our country, we see that ethical approaches to agriculture and food come to the agenda later. However, in Turkey, as in the world, social interest and awareness regarding the ethical aspects of agriculture and food have been increasing significantly in recent years (Taluğ, 2019).

Ethical arguments often focus on harms. What people are concerned about; it is the damage caused or likely to be caused to other living being from a certain action or activity. Whether the harms are justified is a question that ethicists try to answer in several steps: What harm is being considered? How important is it? Who are affected or may be affected? What is the distribution of damages among the various stakeholders? Are those who are at risk of harm from the action in question different from those who could benefit? (Burkhardt, 2005) When ethical choices are made in practice, the consequences of the

action should be well thought out. Because when there are value conflicts, one value is preferred over another and there are sides that will be affected by it. For this reason, the results of the issues related to agricultural activities should be well calculated both in the short and long term and should be decided carefully.

There are several basic ethical theories and the simplest distinction that can be made is; it is possible by considering whether they focus on the morality of the action itself, on the consequences of the action, or on the motives behind it. Another distinction is made by considering deontological theories (related to duty), utilitarian theories (related to obtaining the greatest good for the greatest number of people), and egoism (related to obtaining the greatest good for the moral agent). In practice, people and organizations often try to use a mix of these systems when confronted with ethical dilemmas (Bhardwaj et al., 2003).

Ethical dilemmas in agriculture cover hunger and food security, small family farming and social justice in agriculture, food access-justice in distribution, sustainable agriculture and rural development, biodiversity and biosecurity, agricultural biotechnology use limits and intellectual property rights, animal welfare and many more. Our planet, which is normally in a natural balance with the living things on it, is heading towards an ecological dead end as a result of human activities. Agriculture has a position that both affects and is affected by global climate changes. In the mid-20th century, based on the thesis of scientists that population growth will lead to hunger in the future,

developing resistant and high-yielding seeds to increase agricultural production; agricultural production has increased in quantity with a series of applications including pesticides, herbicides and chemical fertilizers and intensive mechanization. However, as a result of this process called "Green Revolution", the ecological balance has been disturbed by the uncontrolled and excessive chemicals used in production; Soils have become infertile, weeds and harmful insects have increased, differentiated and agricultural chemicals have started to be used more in a vicious circle. As a result, these damages have been observed and proven after a while. In the ongoing process, along with the developments in genetics, transgenic varieties, genetically modified (GM) seeds and crops have started to be produced with biotechnological methods. These products continue to be the subject of discussion today in terms of intellectual property rights and possible risks for human, animal and environmental health. Although it is open to discussion in terms of ethics; despite all these developments that increased agricultural (plant and livestock) production, it is another reality that the problem of hunger in the world has not been solved even today. According to the 2020 data of the "State of Food Security and Nutrition in the World Report" (SOFI), the number of people affected by hunger globally has increased since 2014. The data in the report in question shows that approximately 690 million people or 8.9 percent of the world's population are hungry in 2019, it is stated that the number of people suffering from hunger has increased by 10 million people compared to the previous year and by approximately 60 million people in the last five years. In the light of these updates and data, it is seen

that the world is still far from the zero hunger target set for 2030 (FAO et al., 2020). In 2020, which was under the shadow of the COVID-19 pandemic, approximately 118 million more people faced hunger compared to 2019. Nearly one in three (2.37 billion) people in the world did not have access to adequate food, and an increase of nearly 320 million people in just one year. More than half of the world's undernourished live in Asia, and more than a third live in Africa (FAO et al., 2021). The point that needs to be questioned here is whether the moral responsibility of the problem of hunger is in the agricultural sector or other actors, although the amount of agricultural production is enough to feed everyone. With the increasingly globalized food production systems, the journey of food from soil to table has become more complex. However, access to healthy food and food security are the rights of all people on earth. Although there are millions of people suffering from hunger; The amount of food lost and wasted worldwide is more than 1.3 tons per year. The United Nations predicts that the global population will reach 9.7 billion by 2050 and the demand for food will increase by up to 60 percent (FAO, 2021a).

Today, the concept of sustainability maintains its importance on the agenda. It is now clear to everyone that agricultural production should be carried out in a sustainable way, taking into account the rights of future generations. Actions that will contribute to the climate crisis should be avoided. The share of agriculture in the total ecological footprint of the world countries on a global scale comes after carbon footprint with a rate of 32% (Global Footprint Network, 2017). Striving

for sustainability for the future of agriculture, the agriculture of the future, food security and the well-being of the whole world is not just scientific; it is also an ethical requirement. Because the effect of the practices of any country can affect another country in a very distant corner of the world. For example, Madagascar, an island nation, is about to become the first country to experience famine, driven by climate change, according to a 2021 statement by United Nations officials. As the worst drought in four decades has shaken the country's isolated farming communities, families have begun to eat grasshoppers and cactus leaves to survive. What should draw our attention here; unlike developed countries where millions of tons of agricultural and food products go to waste every year, this island country is exposed to drought and famine even though it does not use fossil fuels (URL-1). Agricultural activities should be carried out without harming wildlife, without destroying forests in order to open up agricultural areas, by preserving the ecological balance, biodiversity, and non-renewable resources such as soil and water. Otherwise, it will not even be possible to save the day with the crops obtained.

Today, agriculture is intertwined with many difficulties and problems in Turkey as well. As in the whole world, with a scientific and ethical approach, while carrying out production activities for nutrition from agriculture, on the other hand, protecting the soil, water and climate; In addition, it is expected to contribute to both social and economic welfare.

Ethical problems/dilemmas in agriculture arise from the tools and methods preferred while carrying out agricultural activities and benefiting from their outputs, rather than the purpose of agriculture. It is clear that the primary purpose of agriculture is production. However, apart from production, it is not only ecological; It is a known fact that it also includes some social factors. Livelihoods, working and living conditions of permanent and seasonal workers, farmers, who constitute the labor factor of agricultural production; issues such as the necessity of improving the production, living and competition conditions of family farms and small agricultural enterprises are important points to be emphasized. Yes, today family farming and the struggle for survival and survival of small businesses are on the agenda of all countries; however, when the current situation is considered, it is seen that there is not enough progress.

It is possible to come across many definitions that meet family farming in the literature. In very general terms, family farming can be defined as "all the activities carried out by the family in connection with agriculture" (Ministry of Agriculture and Forestry, 2014). In general, the life of family businesses is shaped around agricultural activities, their lands, incomes and production potentials vary from country to country, but they are below a certain size and the workforce is often met by family members. Their livelihood and survival depend on plant and livestock production. More than 90% of the more than 600 million farms in the world today are run by an individual or a family and rely mainly on family labor. Looking at the distribution of agricultural lands

around the world, family farms cover approximately 70-80% of agricultural land, and more than 80% of the world's food is produced by family farms. While women own only 15% of farmland, they provide almost 50% of agricultural labor. 90% of fishermen work on a small scale. On the other hand, 33% of forests are managed by indigenous people and local communities (FAO, 2021b). When we look at the agricultural structure of Turkey, it is seen that especially small and medium-sized agricultural enterprises are dominant and production based on family labor continues in most of the country. According to 2017 data, the total employment in our country is approximately 28.2 million people; about 5.5 million of these are employed in the agricultural sector. On the other hand, agriculture sector constitutes approximately two-thirds of rural employment (T.C. Ministry of Development, 2018). When all these data are evaluated, it is seen that small agricultural enterprises and family farms have an important place for all countries in agricultural production on a global basis, regardless of the level of development. In this sense, farmers have a great responsibility in terms of agricultural production. Small farmers and family farming are seen as a key role on a global basis in the continuity and increase of food production to end hunger, reduce unemployment in rural areas, ensure sustainability in agricultural production and protect biodiversity. In this context, the United Nations declared 2014 as the International Year of Family Farming; At the international level and by the member countries, it has been tried to draw attention to the problems and solution proposals of family farming with various activities.

Today, family farming is still on the agenda on a global scale. The United Nations General Assembly declared the Family Farming Decade (2019-2028) in 2017; The Global Family Farming Conference was held in 2019, and the United Nations Global Action Plan was put forward, which will put family farming at the center of the international agenda for a ten-year period. The action plan, which covers a large number of stakeholders around the world, aims to accelerate actions taken to support family farmers, which are important elements of sustainable development (FAO & IFAD, 2019).

When viewed from a wide frame, increasing the productivity and output of small farmers not only increases their incomes and food security, but also stimulates the rest of the economy and contributes to broad-based food security and poverty reduction (Lipton, 2005). In fact, productivity in small family businesses is higher than in large businesses, contrary to what is believed (Shiva, 2016; Özer, 2011). There is an agricultural tradition passed down from generation to generation in family farming, family members are intertwined with agriculture from the moment they open their eyes to the world. In productions that require labor, they are faced with lower transaction costs in the labor force due to the fact that family members generally work. This gives them a productivity advantage over large-scale farms. The productivity of small farmers is also superior when measured in terms of diversity. Biodiversity-based productivity measures show that small farmers can feed the world. It really represents a high level of productivity as its yields consist of

multiple yields of different species used for various purposes (Shiva, 2016).

According to the 11th Development Plan Rural Development Specialization Report, “The main problems faced by family farming in our country; The absence of young workforce or their migration to cities, the lack of tendency to produce and organize for the market, the uneconomical scale of production, and the difficulty of accessing modern agricultural knowledge and agricultural supports can be given as examples” (T.C. Ministry of Development, 2018). By supporting the share of small family farming in production, it is also possible to provide access to resources for the women and young population, who are the disadvantaged segment of the society; their involvement in employment and production provides significant advantages at micro and macro scales. In this way, a contribution is made to the increase in production and employment, and a decrease in the rates of poverty and migration from rural to urban is an expected result (Güresinli, 2015).

Most smallholder farmers face significant difficulties in accessing the market; Strict requirements in modern food value chains can isolate farmers from market mechanisms. Contract farming is seen as a way out for increasing farmers' participation in the market and the modern value chain (Michelson, 2020). Although the contracted production model is currently advantageous in terms of offering the product produced by the farmer to the market, in today's market conditions, it may be possible for industrialists, merchants or market chains to buy the product from the field at prices below its value. It may also result in

buyers having a say in the face of the unorganized producer. Therefore, the contract production model should be carried out under strict control.

In Turkey, the "Research on Urban and Rural Settlement Systems in Turkey (YER-SIS)" covering 37,036 settlements was conducted by the General Directorate of Development Agencies in 2020. According to the Field Study Report on Rural Settlements in Turkey, prepared as a part of this research, livestock and plant production activities come first in order of importance and prevalence of basic livelihoods in rural areas, followed by pensions and social benefits. Considering the labor mobility, 21.1 percent of rural settlements go to another settlement for seasonal construction works, 21 percent for regular work and 11 percent for seasonal agricultural work (General Directorate of Development Agencies, 2020). As seen in this study, agricultural activities appear as the biggest source of income and employment in rural areas, which are critically sensitive to poverty and hunger problems.

In the same report, besides the economic indicators, data on the social structure of rural settlements are also included. According to the presence of active educational institutions, it was determined that 33.93% of the rural settlements in our country had primary schools, 17.62% had secondary schools, 2.17% had high schools and 1.84% had vocational high schools. Considering the existence of health institutions, it was determined that 13.48% had family health centers, 2.74% had pharmacies, and 0.41% had hospitals. In the study, the average distances traveled for basic services were calculated and the services provided from the farthest distance were determined as

hospital, vocational high school and pharmacy, respectively. The service that can be reached from the nearest distance is the primary school. The existence of facilities and infrastructure services that affect the quality of life in rural settlements was also included in the research. Accordingly, the most common facility is grocery-market with 39.24%, followed by social facilities with 12.98% and producer association or cooperative with 11.45%. The most common infrastructure services are adequate mains water infrastructure with 81.19%; mobile internet access with 68.46%, adequate waste water and sewage infrastructure with 46.27%. 32.73% of the people living in rural settlements want social facilities (such as a playground, cultural center, reading hall, village room, gym, etc.), 17.79% a factory/workshop and 9.87% a milk collection center (General Directorate of Development Agencies, 2020). According to the report, it is obvious that the basic needs of people still living in rural areas cannot be fully met and that they have some expectations and demands.

Rural settlements are the places where poverty is most common. With the effect of socio-economic change that started in Turkey in the 1950s, the problems caused by internal migration from rural to urban areas constitute a multidimensional and complex structure (T.C. Ministry of Development). In the 1950s; The phenomenon of migration from rural to urban, which started with the effect of factors such as increasing population, transportation opportunities, and mechanization, accelerated in the 1980s with the youth starting to leave the countryside. According to the latest calculations, the rural population in Turkey has

a rate of 37 percent (SETA, 2019). Especially the leaving of the young people from the countryside results in the aging of the agricultural population in the countryside; It also jeopardizes the future of agricultural production.

In order for those engaged in family farming to maintain their existence in agriculture and rural areas and to prevent rural poverty in general, existing solutions for education, transportation and infrastructure opportunities, reduction of agricultural input costs, market finding and financing problems should be made more effective. By improving living conditions, people should be prevented from leaving their homeland and migrating to the city. In fact, although there are support items and projects allocated by the Ministry of Agriculture and Forestry for small family businesses, young farmers and female farmers in our country, these applications should be continued effectively and with a wider scope.

In addition, first of all, both farmers and farmer candidates should adopt the dignity of the farming profession; It is necessary to disseminate the idea that agriculture is important and valuable to the whole society (T.C. Ministry of Development, 2018).

Even today, when it comes to agricultural problems in Turkey, one of the first things that comes to mind is the existence of smallholder farmers. In fact, what we should see as a main problem should be the inadequacy of organization and cooperatives, infrastructure problems and disadvantages related to production costs. We also have to consider

the social aspect of this problem. Rural areas are emptied when small agricultural enterprises are out of operation. Poverty and unemployment are also increased, as every person who comes to the city cannot be provided with sufficient jobs in the industry and service sectors.

Necessary measures should be taken in order to ensure the continuity and productivity of small agricultural enterprises and farmers, to prevent agricultural lands from becoming idle, to prevent farmers from being lost in the value chain and being cut off from agricultural production.

Apart from the many economic, demographic, environmental and sociological benefits of ensuring the continuity of family farms on a national and global scale; First of all, it is a situation that should be supported ethically. Seeing only the consumer's access to cheap food as the target, without considering the welfare share of farmers and agricultural workers; In a sense, it is not ethically acceptable to allow rural poor people to subsidize the urbanite's desire to provide cheap food. The farmer, the producer, should receive the real reward of his labor and production. Otherwise, it will not be possible to talk about social justice and welfare for farmer families who are struggling to stand up against large and industrial agricultural enterprises due to the inequality of conditions. It is another ethical requirement that the wages and working and living conditions of permanent and seasonal workers working in the agricultural sector are at levels worthy of human dignity.

## **CONCLUSION**

The agricultural sector has a very complex structure. There are many stakeholders that it affects and is affected by. In this respect, agriculture, which is perhaps the most indispensable activity in the world, should be maintained within an ethical framework. For example, it is not possible to talk about agricultural ethics without honest technical knowledge (Dundon, 2003). In addition to scientific studies, honest and responsible production is one of the requirements of agricultural ethics. Resources that were used as if they would never end are now at the limit. It is essential that everyone who is involved in the agricultural activity and directs the agricultural sector in some way observes these.

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## CHAPTER II

### MICROGREEN: A GENERAL REVIEW, THE IMPORTANCE AND CHANCE OF ITS DEVELOPMENT IN TURKEY

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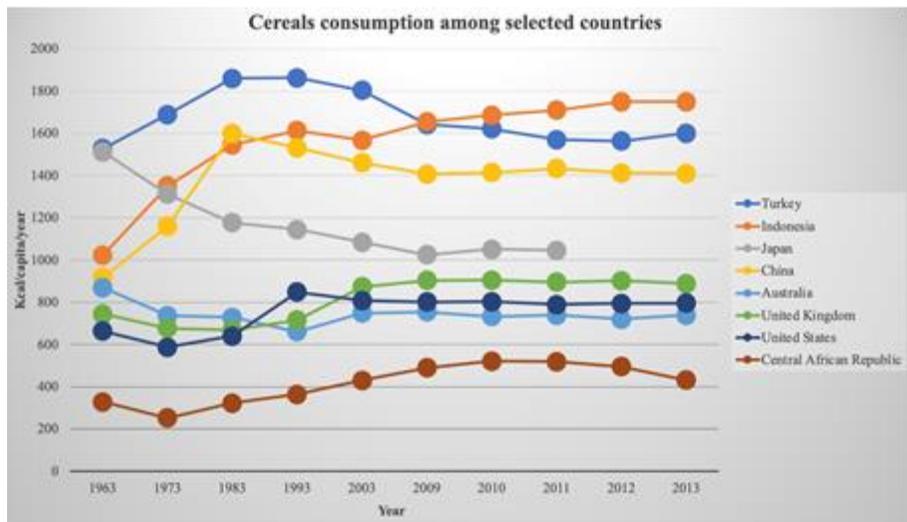
## **INTRODUCTION**

Agriculture, as the main sector supplying vegetable food. It has to act swiftly for quantitative and qualitative increase in yield by developing innovations in vegetable cultivation techniques and processing. Rising global urbanization pose serious threats to vegetables production and food supply in many countries, especially the developing countries over the next decades. The people have observed extreme complex risks in their vegetable supplies in large urban areas.

Microgreens, the edible young vegetable crop plants, are popular in recent years, world over. These could be used as a functional food due to rich nutrient compositions. There is limited information about microgreens cultivation and utilization in Turkey. This paper offers an idea of microgreen production in urban areas that can withstand limitations of current urban farming practices. These practices could also act in transformation of the concept of agriculture and advance possibilities in reducing risks of vegetables supply chain. Proper business planning, study encouragement and organization with the help of academics and research organizations would offer opportunities for urban agriculture. This will offer newer shorter food chains and better opportunities for every one.

## 1. GLOBAL FOOD CONSUMPTION TREND

A balanced, healthy diet including sources of protein as milk, white and red meat and their products legumes oil seeds, fresh vegetables, fruits, bread and cereals should be consumed in a sufficient quantity. Cereal grains occupy an important place in basic food nutrition in many countries of the world. However, demographic, lifestyle factors and other factors as income and price among the countries affect food consumption and nutritional quality in agreement with Akbay (2018). The differences in cereal foods consumption of some countries is shown in Figure 1.

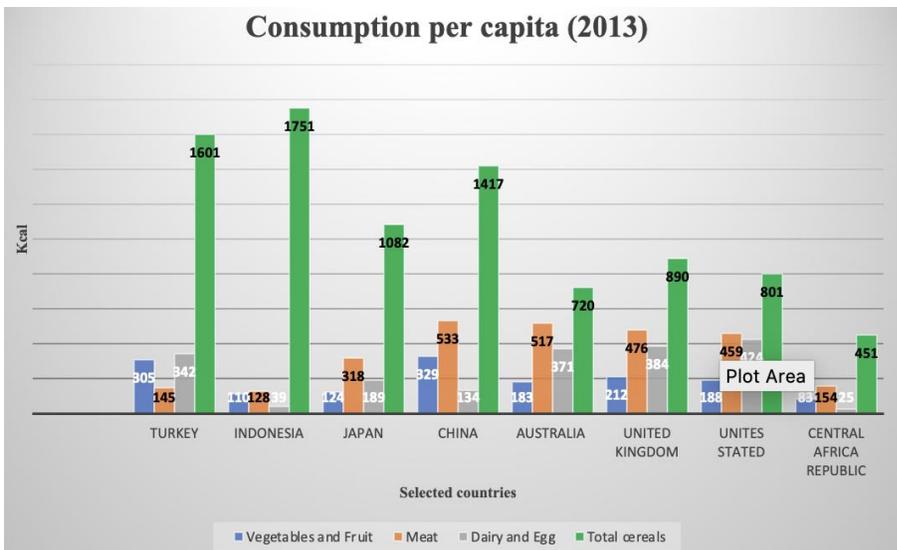


**Figure 1:** Total Energy Intake of Cereals Among Selected Countries (Our World Data, 2020a)

People who live in the Middle Eastern and Asian countries have habit of consuming high amount of cereals compared to those living in the industrialized countries like United Kingdom and United States.

However, there is a trend of using reduced cereals in Japan, China and Turkey since last few years.

Cereals have an important role and contribute ~70% of energy intake in the developing countries of Africa and parts of the Asian continent, while they supply ~30% of energy intake in industrialized countries (Figure 2). It has been estimated that the share of cereals will tend to decline slowly from 54% in 2001 to 49% in 2030 and 46% in 2050 (Alexandratos et al., 2006).



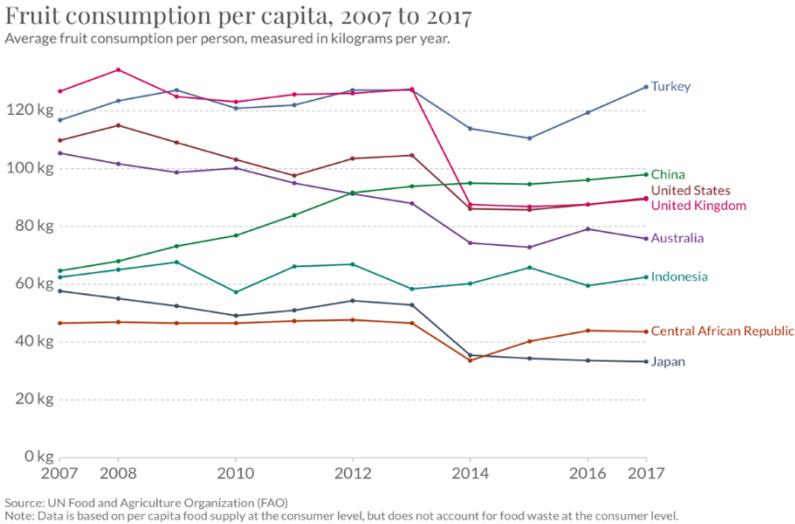
**Figure 2:** Nutritional Varieties of Food Consumption Among Selected Countries (Our World Data, 2020b)

Level of income has also an impact on food consumption in a country. Higher fat diets could be correlated to rising income. Increasing consumption of animal-based foods and processed foods is one of

impact of rising incomes or lower prices. Those who also have good education can also help to select to focus on adopting a healthier lifestyle. Fewer food choices and less nutritional education has also resulted in fewer options for the poor. It has been known that better quality diets, healthcare, lower morbidity and mortality from infectious diseases and lower risks of obesity are reflections of rising income in the industrialized and rich countries like the USA and the UK (Marmot, 2001). Whereas, comparatively high prevalence of obesity is a common feature in poor individuals of low-income countries compared to wealthy individuals (Templin et al., 2019). Figure 2 shows dietary composition of four commodity groups among some selected countries during 2013. The selected industrialized and developed countries with high income like USA, UK and Australia, get higher energy intake from animal-based foods. Similarly some other selected countries and Japan as developed country also tend to consume lower amount of cereals compared to majority of the Asian countries. There is increased trend of using animal-based foods in some selected urban areas of some developing Asian countries like China and Turkey, in upper middle income groups. Akbay (2018) has pointed out that food consumption data of Turkey (1963-2013), predicts higher fat and protein consumption in relation to income elasticity and increasing average household income levels.

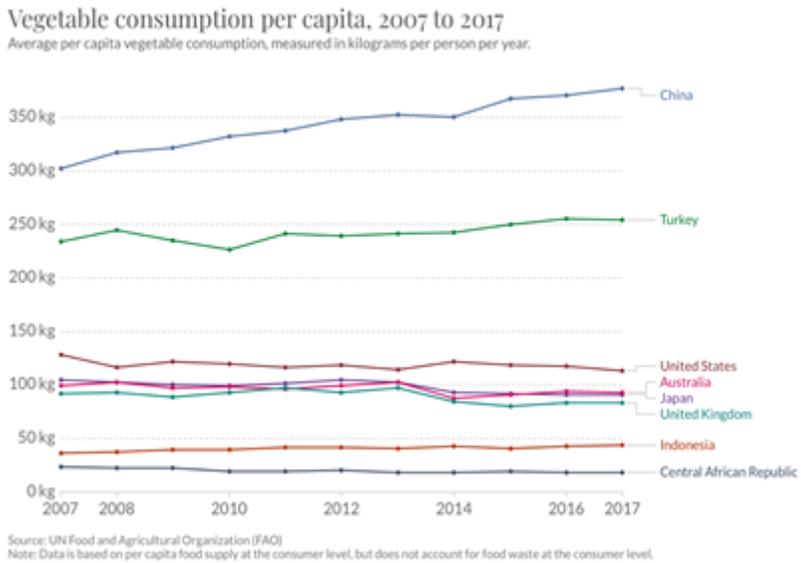
Although fruit and vegetables consumption per capita (kcal) in developed countries is higher compared to these types of spendings in developing countries, yet there is a general trend of decreased total fruit

and vegetables consumption during last few years. Figure 3 showed that trend of fruit consumption in developed countries like USA, UK, Australia and Japan tend to fall in last ten years (2007-2017). As a developing country, China has begun to raise fruit consumption in the last decade, Turkey noted a trend to fall in fruit consumption in 2015 and rise in 2016. It might be due to falling employment in agriculture correlated to rural-urban migration that lead to decreasing in agriculture and food production. It has been expected that changing rural-urban population structure have an impact on types of employments. This is linked with the structural transformation process: with increased urban population shares in employment. This in turn tends to shift from agriculture towards industry/manufacturing, or other services in agreement with Michaels et al. (2012). Covid -19 pandemic has seriously affected life styles and living habits of people world over. The pandemic also caused it difficult for the people in controlling and securing strategic goods and services due to number of reasons including lesser availability of migrant labourers; who have a large contribution in plucking, packing and providing logistic services. These services are facing partial disruption in global supply chain in agreement with Siche (2020) and Richards & Rickard (2020). Christiaensen et al. (2021) has also emphasized improvement and strengthening of automation, digitization trends and less reliance on migrant labourers to improve trade in developed countries during the Pandemic period.



**Figure 3:** Fruit Consumption Per Capita Among Selected Countries in The Last Few Years (Our World Data, 2020c)

While vegetable consumption has also tended to fall in developed countries, it tends to rise in China and Turkey in the last ten years between 2007-2017 (Figure 4). The stable vegetable consumption has been shown in the least developed country of Central African Republic. Terin et al. (2019) noted that the supplying fresh and frozen of fruit and vegetables in Turkey is affected by socio-demographics and economic factors. These are expressed by increasing in age, educational level and income of household head, married and male household head, household head working status, number of adults and households who get help in-kind or cash from the government or private sectors.



**Figure 4:** Vegetable Consumption Per Capita Among Selected Countries in The Last Few Years (Our World Data, 2020d)

## 2. URBANIZATION

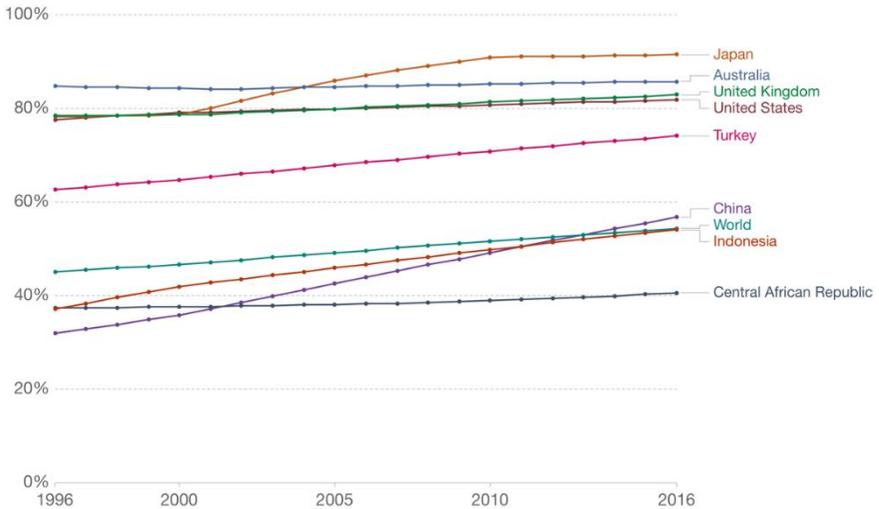
The urbanization can lead to difference of food consumption patterns between urban and rural areas in agreement with Popkin (1999) and Regmi & Dyck (2001). Urban residents tend to eat more meat, fruits and vegetables. It differs from rural residents who tend to consume more carbohydrate in form of cereals, tubers and roots. Mottaleb et al. (2018) points out that wheat, pulses and fish are more consumed and popular among both rural and urban households in Bangladesh. They further notice a reducing trend of rice consumption in from 2015 onward period that will be significantly reduced in urban areas in 2030s.

At the same time, a higher energy intake through animal based foods in form of fast-food consumption by lower spending urban jobs has induced higher risks of cardiovascular or chronic diseases compared to the rural people. Mendez & Popkin (2004) has suggested that this is mainly due to the use of imbalanced diets without balanced use of fibre, vitamins and minerals. This trend has induced problems in them and will appear as the main future problem of the developing countries.

The approximately 55% of people in the world who live in urban areas have been reported. It is estimated by UN that more than two-third of world's population will live in urban areas by 2050 (Meredith, 2020). It has been reported that more than 80% of population in high income countries (Western Europe, the Americas, Australia, Japan and the Middle East) and 50-80% of upper-middle income countries's population (Eastern Europe, East Asia, North and Southern Africa and South America) live in urban areas (Figure 5). The majority population of low-lower middle income countries are still living in rural areas. The increasing urban population in developing countries is projected higher than in developed countries over the next century.

The accelerated urbanization will affect dietary changes and change in consumer preference. Correlated to negative impact on food consumption, people have to consider for food safety and organic foods. As an alternative future food, the nutritional varieties must have balanced fibre, protein (amino-acids), vitamins, minerals, supplements; with reduction in fat and saturated fat contents, sugar, salt, alcohol; with

substitution of these with variety of fat, salt and sucrose replacers, (Gordon, 1998). The smart future food could be obtained from neglected and underutilized species (NUS) which have essential micronutrients, protein, energy and fiber (FAO, 2018).



Source: OWID based on UN World Urbanization Prospects 2018 and historical sources (see SourcesOurWorldInData.org/urbanization • CC BY Note: Urban areas are based on national definitions and may vary by country.

**Figure 5:** Urbanization Over 20 Years (1996-2016) Among Selected Countries (Our World Data, 2020e)

To respond urbanization trend in the future, both the agriculture sector and healthy food will have an opportunity to be considered by individuals and governments world over. Urban agriculture could be considered as an alternative of food supply in the present and the future. According to World Health Organization (WHO), global dietary guidelines among countries pushes consumption of variety of foods, including fruits and vegetables, legumes or nuts, variety of animals

based food (dairy, red meat), healthy fats and oils with limited sugar, fat and salt consumption (Herforth et al., 2019).

### **3. URBAN AGRICULTURE AND ITS IMPACTS**

Urban agriculture or urban farming, can be defined as realization of agricultural activities, cultivating plants and raising animals for food, within and around urban areas called intra and peri-urban agriculture, respectively (FAO, 2007). Urban agriculture roles between developing countries and developed countries are still debated due to these characteristic, along with its positive and negative impacts (Hamilton et al., 2013; Orsini et al., 2013; Mok et al., 2013).

Thebo et al. (2014) distinguished that peri-urban agriculture is located within 10 and 20 km of urban agriculture boundary. The distinction elements between urban agriculture and ordinary agricultural activity are agricultural areas, economic activity types, types of production system, target and quantity of the end products. These range from food production by low-income families for their own consumption to community and hobby gardens or large-scale agricultural enterprises (Mougeot, 2000a; Thebo et al., 2014). There are differences in household activities for agricultural purposes between global North and global South. McClintok (2010) noted that rooftops, balconies, vacant lots, road medians and parks are usually used for agricultural purposes. Whereas, undeveloped lands, marginal lands and community plots are used for farming of household food consumption and empty areas of post-industrial landscapes are used for agricultural purposes (Azunre et al., 2019).

Urban agriculture might be an alternative to both food security and self-provisioning led to improve quality of urban diets. This will supply more fresh fruit, vegetables and animal products for urban areas, especially for poor households (Armar-Klemesu, 2000; Garret, 2000, Kortright & Wakefield, 2011). Quantity and quality of fruit and vegetables containing vitamin, minerals and fibers supported by organic urban horticulture system have impact on body health. Studies conducted by Boffetta et al. (2010) and Reiss et al. (2012) approve it and confirm that increasing consumption of fruit and vegetables could be one of alternative to prevent rising cancer cases. Looking at cancer or other chronic diseases avoidance effect could be correlated to body immunity ability againts virus. Healthy body with health immune system will keep, response and recovery faster from foreign material entering the body.

Urban agriculture has also impact on household income (urban economic) and ecological systems and could support factors responsible for sustainability of economic, social and environment (Azunre et al., 2019). The women who do not have a work can perform home scale agricultural activities. Orsini et al. (2013) have noted that 65% of urban farmer are women. The product of household agriculture can be used to domestic consumption that can reduce household food cost or sold to others. Other previous study by Mougeot (2000b) present that maintaining small livestock can enhance 60% of over quarter household income in Cairo, Egypt.

Aşlıoğlu & Çay (2015) explained that environmental impacts of urban agricultural areas are changing urban climate, improving air quality, preventing soil erosion and preserving biological diversity. Despite having positive impact to health, economic, social and environment, urban agriculture also raises some problems to environment due to using public lands and sharing of air, water and soil resources among humans and animals. There is need to be consider environment, agroecological conditions and household characteristics of urban areas supported by national, regional and local policies such as access of land, credit, training and counseling in building urban agriculture.

### **3.1. Urban Agriculture in Turkey**

It is noted that Istanbul, Turkey took rank 17th with 1.75% yearly growth rate between 2006-2020 (Orsini et al., 2013). Kutlar (2014) also pointed that 8.7% population of Turkey live in rural and 91.3% live in urban areas. The condition has impacts on agriculture sectors notably agricultural production in relation to food supply. The main reason for under employment in agricultural sector is utilization of agricultural lands for housing, industrialization, erosion, and high use of chemicals in agricultural practices. Downsizing through inheritance is also an important factor that influence agricultural production.

High intake of plant based foods (cereal, fruits and vegetables), olive oil (mono-unsaturated fat source) and fish and utilizing variety of diverse local plants and animal species are a characteristics of traditional Asiatic Mediterranean diets including Turkey (Garcia-

Closas, 2005; UNESCO, 2010; Dernini et al., 2012). Although Turkey is the largest producer of fruit, vegetables and nuts among the Middle Eastern countries and the 7<sup>th</sup> largest in the world, yet it imports some categories of fruits and vegetables.

### **3.2. Urban Agriculture Product for Supplying Plant-Food Nutrition**

According to Armar-Klamesu (2000), staple crops such as maize and cassava, fruity, leafy and rooty vegetables are products of urban agriculture. Similarly, Orsini et al. (2013) emphasized excess of urban horticulture due to nutritional value, livelihoods and income addition. It is about 100 million people of the world who are involved in urban agriculture activities which are increasing yield potential up to 50 kg/m<sup>2</sup> for the most vegetables production (Eigenbrod & Gruda, 2015).

There are four types of traditional urban horticulture systems (Orsini et al., 2013):

- a. Allotment and family gardens,
- b. Simplified extensive systems,
- c. Shifting cultivation, and
- d. Intensive systems with addition of innovative systems such organoponics and simplified soilless cultures.

Eigenbrod & Gruda (2015) also emphasized the importance of organoponic and others low-input system to produce sustainability and security in food supply over next years. Urban agriculture with recent

innovative technologies could contribute to food security and environment sustainability in the future (Armanda et al., 2019).

Microgreens could be developed as one of low-input system of urban agriculture product for academic or bussines purposes in the present and future. The Good Agricultural Practices (GAPs) and Good Handling Practices (GHPs) are needed to develop existing microgreens industry (Riggio et eal., 2019).

## **4. MICROGREENS: DEFINITION, NUTRITION, USAGE AND CULTIVATION**

### **4.1. Definition**

Micgrogeen is different with sprout and baby green. Microgreens, a term used to explain an edible green in their juvenile growth stage, are young vegetables green or called as salad crop shoots that have three parts; a central seed, a cotyledon or leave and a pair of young true leaves which could be consumed (Lee et al., 2004; Choe et al., 2018; Paradiso et al., 2018). These parts differ from sprouts containing seed, shoot and root system which are consumed. Whereas, baby greens, also called baby leaves, are defined as leafy vegetables harvested before plant maturity with their first set or more of true leaves (Jaden, 2014). Shortly, it can be expressed that youngest to oldest age and smallest to largest size are sprouts, microgreens and baby greens, repectively.

Migrogreens are more popular and their demand is increasing over last few years especially in the developed countries due to their potential as a functional foods containing rich nutrition and short production cycle

compared to mature vegetables. Similar to Samuolienė et al. (2012) who noted microgreens as functional food to promote health or prevent diseases. In addition, microgreens are also notable CEA (controlled environmental agriculture) crops due to quick harvest and high market value (Gomez et al., 2019; Royte, 2020).

## **4.2. Microgreen Crops**

There are many of crops species commonly grown as microgreens around the world. Bumgarner & Metallo (2017) classified common vegetables and herbs can be used as microgreens based on suitable seasons, while Morton & Strech (2018) categorized by cycle of crops (Table 1). In addition, Turner et al. (2020) mentioned common families grown to microgreens are Alliaceae, Amaranthaceae, Apiaceae, Asteraceae, Brassicaceae, Cucurbitaceae, Fabaceae, Lamiaceae, Oxalidaceae, Poaceae, Polygonaceae and Portulacaceae.

The 25 microgreens varieties have been commercialized in the USA. The microgreens include arugula, bull's blood beet celery, China rose radish, cilantro, garnet amaranth, golden pea tendrils, green basil, green daikon radish, magenta spinach, mizuna, opal basil, opal radish, pea tendrils, peppergrass, popcorn shoots, purple kohlrabi, purple mustard, red beet, red cabbage, red mustard, red orach, red sorrel, sorrel and wasabi (Xiao et al, 2012).

**Table 1:** Common Crops Used to Grow Microgreens

<b>Suitable Seasons</b>	<b>Cycle of Crops</b>
<b>Cool-season vegetables</b>	<b>Fast growing vegetables (7-14 days)</b>
Arugula, beets, broccoli, cabbage, carrot, cress, endive, kale, lettuce, mustard, mizuna, pea, pak choy, radish, Swiss chard, turnip	Cabbage, corn, cress, kale, kohlrabi, mustard, radish
<b>Warm- season vegetables</b>	<b>Slow growing vegetables (15-20 days)</b>
Amaranth, sweet corn	Amaranth, arugula, beet, carrot, Swiss chard, scallion
<b>Herbs</b>	<b>Slow growing herbs (15-30 days)</b>
Basil, cilantro, dill, fennel, marjoram, parsley	Anise, basil, cilantro, dill, fennel, parsley, saltwort, shisho, sorrel

### 4.3. Nutrition and Bioactive Components

Microgreens, as functional food, contain rich bioactive food components such as antioxidants (carotenoids), phenolics, vitamins (phyloquinone, tocopherols and ascorbic acid) minerals, fiber and other micronutrients. These are linked to help optimization of health and reduction or prevention of certain disease risks compared to mature greens or seeds (Janovska et al., 2010; Xiao et al., 2012; Mir et al., 2017). The differences level of essential micronutrients and bioactive compounds might be shown on different growth stage of plants depending on crops used. A study on 25 commercially microgreen varieties in the USA conducted by Xiao et al. (2012) noted higher nutritional densities on cotyledon leaves of microgreens compare to their mature leaves.

The differences phytonutritional components of four accessions of amaranth on different stage: sprouts, microgreens and fully grown was also noted by Ebert et al. (2014). Lopez et al. (2018) noted the high carotenoid of lettuce, carrot and zucchini sprouts and microgreens compared to their baby leaves and the high total polyphenol on carrot and zucchini sprouts and microgreens compared to baby leaves. The high bioaccessibility value of phenolic compounds percentage as flavonoids (8%), tyrosol deviates (9%) and lignans (14%) have been noted on *Brassica* microgreens (kale, cabbage, kohlrabi and purple radish) (Tomas et al., 2021).

#### **4.4. Usage**

Microgreens can be consumed as cold dishes or rawa such as salads, smoothies and sandwiches, as a consequence no losses of micronutrients in agreement with Ebert et al. (2014). In addition, although microgreens can be served as garnish on top of hot dips or dishes, they should not be heated due to quick deterioration when cooked (Tamilselvi & Arumugam, 2018). They also noted that the unique flavors and aroma are brought from a rough chop of microgreens. A review by Sharma et al. (2020), a whole array of microgreens leaf color, shapes and varieties with a great taste compared to sprouts.

#### **4.5. Cultivation and Shelf Life**

Microgreens are easy to cultivate due to low-input systems with indoor farming model. Microgreens growth is affected by many factors. Ebert

(2012), Xiao et al. (2012) and Ebert et al. (2014) declared that microgreens can be cultivated in the soilless media or soil substitutes with little fertilizer or without fertilizers and pesticides since seed nutrient content is enough for seedling or young crop growth. However, the various types of organic fertilizers like compost or vermicompost can be applied to increase microgreens yield (Murphy et al., 2010). The cycle of microgreens growth is within 7-28 days on the light and growth media for growing seeds with ~ 2 inches or 5 cm tall harvested plants depending on crops used (Di Gioia et al., 2017; Treadwell et al., 2020). It differs from sprouts that usually require the dark or low light, high humidity and temperature without growth media, fertilizer and agrochemicals for growing seeds and their growth cycles is shorter (4-10 days) with less than 2 inches plant high (Di Gioia et al., 2017; Lopez et al., 2018; Treadwell et al., 2020). Whereas, the light and either soil or soilless system are generally needed by baby leaves for their growing which has 20-40 days with 3-4 inches (7.5-10 cm) plant length before harvesting.

Microgreens, as fresh product, cultivated by indoor farmers techniques using soil-free horticulture techniques like hydroponics, aquaponics, aeroponics, or growing on mats and soil alternatives may cause contamination in agreement with Alegbeleye et al. (2018). They declared that soil, irrigation water, wildlife, insects, livestock, pets, or soil amendments like fertilizer can be a medium transferring pathogens to plant. The study conducted by Rönnqvist et al. (2014) reported that human hands are main reservoir of pathogens transferring to foods.

Tamilselvi & Arumugam (2018) emphasized that development of pest and disease could be reduce by using sterilized growth media and bottom watering to keep their stem and leave dry. It is supposed that human hand is also able to led to the contamination of microgreen products.

Microgreens are often equated with sprouts characteristics which linked to food-borne illness. Despite some product of microgreens had been reported as subject recalls related to *Salmonella* and *L. monocytogenes* contamination since 2016, no foodborne illness had been reported (Turner et al., 2020). However, Effects of some diseases like *Salmonella* or *L. monocytogenes* contamination are reported on some microgreen products that depress their quality (US Food and Drug, 2016; 2017; 2018, and Canadian Food Inspection Agency, 2018a; 2018b; 2018c). There are also many reports that mentioned different pathogens or parasits contamination on radish sprouts in Japan (Michino et al., 1999), mung bean sprouts in Norway (Robertson et al., 2005) and bean sprouts in Netherland (Reuland et al., 2014). Alegbeleye et al. (2018) also reviewed that some crops as basil, berries, green onions, lettuce, melons, parsley, spinach, sprout and tomato have greatest risks to carry human pathogenic contaminants.

Işık et al. (2020) studied effect of irrigation water and growth media on lettuce and radish microgreens. They noted that irrigation water had no significant effect and the higher *E. coli* contamination was noted in perlite than in peat moss. Radish microgreens have more risks of contamination compared to lettuce microgreens. Other study on

sunflower microgreens with two types of soil-free growing medium (SFGM) (sphagnum peat with vermiculite and Biostrate® biodegradable mats) inoculated by *S. Javiana* and *L. monocytogenes* FSL R2-584) also concluded that survival of pathogens might be partially related to growth media as root environment of microgreens which support nutrients for human pathogens development (Misra, 2020).

The light and temperature requirement of microgreens growth are different depend on types of plant. Generally, the moderate light and temperature are required on microgreen crops. It is notable that plants need at least 6 hours or more of sunlight a day to optimize microgreen growth under home cultivation in agreement with Bumgarner and Metallo (2017). The differences on humidity and light between sprouts and microgreens cultivation might become reservoir of pathogens contamination in agreement with Ebert (2012) and Ebert (2013). Tamilselvi & Arumugam (2018) revealed that seedling and harvest techniques of microgreens could be considered. Seeds of microgreens could be cultivated in rows or as a broadcast method and too much thick could be avoid due to high risk of various diseases. To reduce contaminated product could be prevented by height cutting when microgreens harvested.

## **5. MICROGREENS: CASE OF TURKEY**

Microgreens are newly used in Turkey. They are rarely used at homes restaurants with innovative kitchens. A report in 2018 by Üstkanat

showed that 80% of Lal Micro Farm's customers were never successful in microgreens farming.

### **5.1. Commercial Microgreens**

All of microgreen producer or companies in Turkey sell their products to their customers through their own websites and rarely through mega supermarkets in shops located in areas where rich people live. Can Bahce company has commercialized microgreen product of radish, red cabbage, basil, red beet, coriander, rocket (aragula), cress, broccoli, sunflower, pea, amaranth, corn, wheat, dill and mustard in Turkey such. There are amaranth, rocket, basil, broccoli, cabbage, cauliflower, chives, cress, ground cabbage, kale, leek, mustard, green and red radish that have been sold by Micro Farm company. Grains such as rice, oats, wheat, corn and barley and legumes such as chickpeas, beans and lentils can sometimes be grown as microgreens.



**Figure 6:** Microgreen Products of Can Bahce Company (URL-1)

## **5.2. Researches and Development of Microgreen in Turkey**

There is limited information on microgreen academic studies conducted in Turkey. Işık et al. (2020) had grown microgreens of lettuce and radish in different growth medias. It was noted that perlite showed high contamination of *Escherichia coli* compared to peat moss after 28 days of culture. There are many factors affecting contamination on microgreens growth as seed source, watering and growing media and conditions.

The rich agricultural or horticulture sector has some limitations in Turkey. The use available plant genetic resources is needed to improve productivity and sustainability as microgreens development. There are

281 accessions belonging to Leguminosae (total of green bean, pinto bean, pea and broad bean) and 222 accessions belonging to Brassicaceae (total of kale and cabbage) collected as vegetable based genetic material in the Black Sea Region (Balkaya, 2009). Balkaya & Karaagac (2006) also noted the number accession of Brassicaceae collected at the Aegean Agricultural Research Institute (AARI) was 389 accession (total of *Brassica* spp., *B. oleracea*, *B. rapa*, *B. napus*, *B. campetris*, *B. nigra* and *B. cretica*). The 295 of accession number of Compositae, lettuce (*Lactuca sativa*) had also been noted as vegetable genetic resource stored in AARI (Tan, 2009). These lettuce consist advance cultivar, landrace or traditional cultivars and wild species. There is also known edible legume of lentils in Turkey which is one of agricultural export product. The red and greens lentils of local cultivars which have small to large seed are also cultivated by farmers. Beside as exports product, mentioned that the five wild species had been discovered in Turkey as *Lens ervoides*, *L. montbretti*, *L. nigricans*, *L. odemensis* and *L. orientalis*. It had also been founded primary progenitor of pea and know as wild and weedy species like *Pisum humile* and *P. elatius*. There is need to look for breeding of plants used exclusively for microgreen farming.

The identification on nutrition of these mentioned plants which could be consumed by human is needed at first step. Subsequently, reseaches in seed technology and plant breeding might be needed to support production of best quality and quantity of seed that could be used as seed source of microgreens. There are also many types of microgreens

growth media. It will also provides the large opportunity to study utilization of various media and other appropriate growing factors to prevent contamination of foodborne pathogens during production.

## **CONCLUSION**

Microgreens could be an alternative of energy intake which provide rich of nutritional components, flavor, texture and color linked to reduce malnutrition and promote good human health in the future. These will enable urban and peri-urban areas to participate in the practice with involvement of minimum land area which often is limiting factor in urban agriculture. There is limited information of microgreens studies conducted in Turkey. Putting forward this concept will will open the opportunities to study microgreens using indigenous crops of Turkey or introduced to Turkey with health benefits due to their nutritional components. There will be a need to carry out studies on selected crops to evaluate nutritional quality of microgreens, in this context. Growth stages, best time for harvesting, effects of various growth media, growth conditions, packaging, storage, cooking and processing techniques during production based on the consumers preferences will be desired for food safety. These studies will provide guideline about risks and benefits of microgreens as an alternative and innovative crop in urban agriculture and will encourage cheap and simple homescale microgreens farming.

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## **CHAPTER III**

### **TULIP BULB PRODUCTION IN TURKEY**

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## **INTRODUCTION**

The ornamental plant trade accelerated at the beginning of the 20<sup>th</sup> Century and turned into a significant market after the Second World War, within well developed and developing countries. Especially in the last 50 years the change and expansion became rapid making a notable contribution to the world's economies. Today, plants are no longer seen as ornamental but are instead integral to people's social lives and living spaces supported by an increased in the variety of styles offered by the market.

The related market also covers natural flower bulbs which are scientifically classified as geophytes. Geophytes are herbaceous plants that store nutrition underground in organs which take the form of bulbs, corms, and rhizomes, and contain substances essential to the development of a new plant. Typical examples would include tulips, hyacinths, irises, lilies, and daffodils (Zencirkiran, 2002).

Among them, the tulip has been one of the most striking ornamental plants, with a 400-year long cultivation history. The centuries old intense artificial selection process has generated many visually attractive tulip varieties. The obtained cultivars with different colour, shape and lengths have since been used in landscapes and in floriculture.

Today, the bulb production for flowers covers an area of 43,000 hectares in the world. The tulip holds its place as the most popular bulbous plant, in terms of the production (Podwyszynska & Sochacki, 2010). More specifically tulip production is significant, taking place in some 15 countries worldwide, with the largest production area being in the Netherlands with 10,800 hectares (88%). This is followed by Japan (300 hectares, 2.5%), France (293 hectares, 2.4%), Poland (200 hectares, 1.6%), Germany (155 hectares, 1.3%) and New Zealand (122 hectares, 1%). The Netherlands produces 4.32 billion tulip bulbs, of which 2.3 billion (53%) are used as the starting material for the cultivation of cut flowers, and 1.3 billion of these (57%) are grown as cut flowers. The rest are exported to countries within the EU (0.63 billion) and outside the EU (0.37 billion). In France a substantial part of the production is controlled by Dutch companies and used in the Netherlands for early planting (November-December). The tulips cultivated in the Southern Hemisphere are scheduled for autumn flowering (October-December) in the Northern Hemisphere and exported to the US, the Netherlands, Japan and Canada (Buschman, 2005).

The Netherlands receives hundreds of thousands visitors every spring particularly at Keukenhof flower garden and the Bollenstreek tulip fields which generates a significant input for the county's overall economy (Figure 1 and Figure 2).



**Figure 1:** Keukenhof flower garden in the Netherlands (Original by Salman)



**Figure 2:** Keukenhof Flower Garden in the Netherlands (Original by Salman)

## **1. THE HISTORY OF TULIP**

The tulip, a symbol of love and imagination, originates from the Central Asia. The plant with no traceable scent yet striking colour and shape spreads widely from the Pamir mountains in the north to Kashmir in the south, up to an elevation of 4000 metres, and, from China in the east to Turkey and the Caucasus in the west (Hoog, 1973; Pavord, 1999; Doobs, 2003; Eker et al., 2014). The tulip flower became so prominent in the Ottoman Empire, that it gave its name to an era, shaping not only the horticulture but also the arts, literature, and architecture of that time.

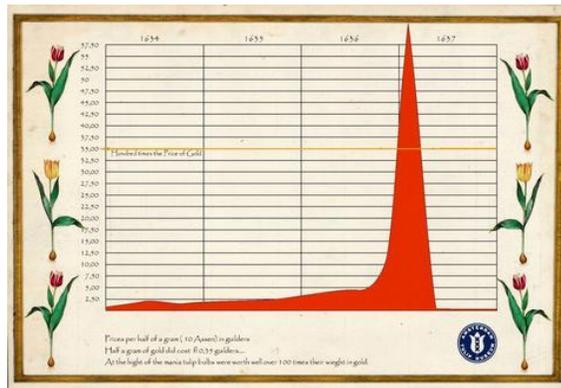
The Turks have cultivated this perennial plant since the 11<sup>th</sup> century. The plant was unknown during the Roman and the Byzantian times, therefore it is assumed that the tulip was spread to the Anatolian peninsula by the migrating tribes from the Central Asia. While the Seljuks interacted with the plant it was less widely so than the Ottomans. The documents indicate that the dominance of tulip begins with the rise of the Ottoman Empire. Sultan Murad IV, as a keen horticulturist, cultivated and registered 56 varieties of tulip. For this purpose, The Flower Academy (Encümeni Danış-i Sukufe) was established to record the increasing number of tulip cultivars. This establishment registered nearly 2000 tulip varieties (Pavord, 1999). Therefore, it was the Turks who cultivated and hybridised the plant first in its history (Baytop, 1992, Pavord, 1999, Alp et al., 2013). These original tulip species, known as Ottoman tulips, bore strikingly elongated spear shaped petals. On the other hand, the later emerging

European varieties, which lacked this essentially elegant characteristic, became referred to as crude tulips among the public. Today the search continues to find the lost Ottoman tulip which traces back to the original species.

The European history of the tulip plant starts in the 15<sup>th</sup> century with the Flemish diplomat to The Ottoman Empire, Ogier Ghislain de Busbecq. He first sent some specimens to Carolus Clusius, his renowned botanist friend at the University of Leiden (Van der Sloot 1994; Pavord, 1999) (Figure 3). The tulip which was planted in 1593 in the university's botanical garden, was cultivated rapidly and finally gave its name to the phenomenon known as Tulip Mania which reached its peak in 1637. It was during the Dutch Golden Age that the bulb prices reached an equivalent of a high market house prices in Amsterdam. Similarly, while a skilled artisan earned 300 guilders annually, the price of a single tulip bulb could range between 3000 and 12000 guilders (Figure 4).



**Figure 3:** Keukenhof Flower Garden in the Netherlands (Original by Salman)



**Figure 4:** Tulip Value Increase during the Tulip Mania (Anonymous, 2021a)

Eventually demand for tulip bulbs led over production, sudden loss of value loss and bankruptcy (Christenhusz et al., 2013). The relative popularity of the tulip came to a rapid end.

## 2. GENERAL CHARACTERISTICS OF TULIP PLANT

Tulips (*Tulipa* sp.), as a herbaceous and bulbous perennial, belong to the Liliaceae family with 75 species and 4 subspecies. It is believed that the word tulip comes from turban. The hybridised cultivars are often used in floriculture and in landscape design. The most preferred cultivars are obtained from *Tulipa gesneriana*. Although tulips can be bred from seed (Figure 5 and Figure 6) bulb production is the most common way of propagation for commercial purposes. Seed production is often used to obtain new cultivars and the breeding process can take up to 5 years (Salman et al., 2015). Releasing a new cultivar into the market may require several decades. Tulips planted in autumn flower in spring. They are often classified on flowering time or petal shapes as single early, double early, triumph, Darwin hybrid, single late, lily-

flowered, fringed, viridiflora, rembrandt, parrot, double late, kaufmanniana, fosteriana, greigii and multiflowering (Figure 7 and Figure 8). The plant height of the cultivars varies between 10 and 80 cm.

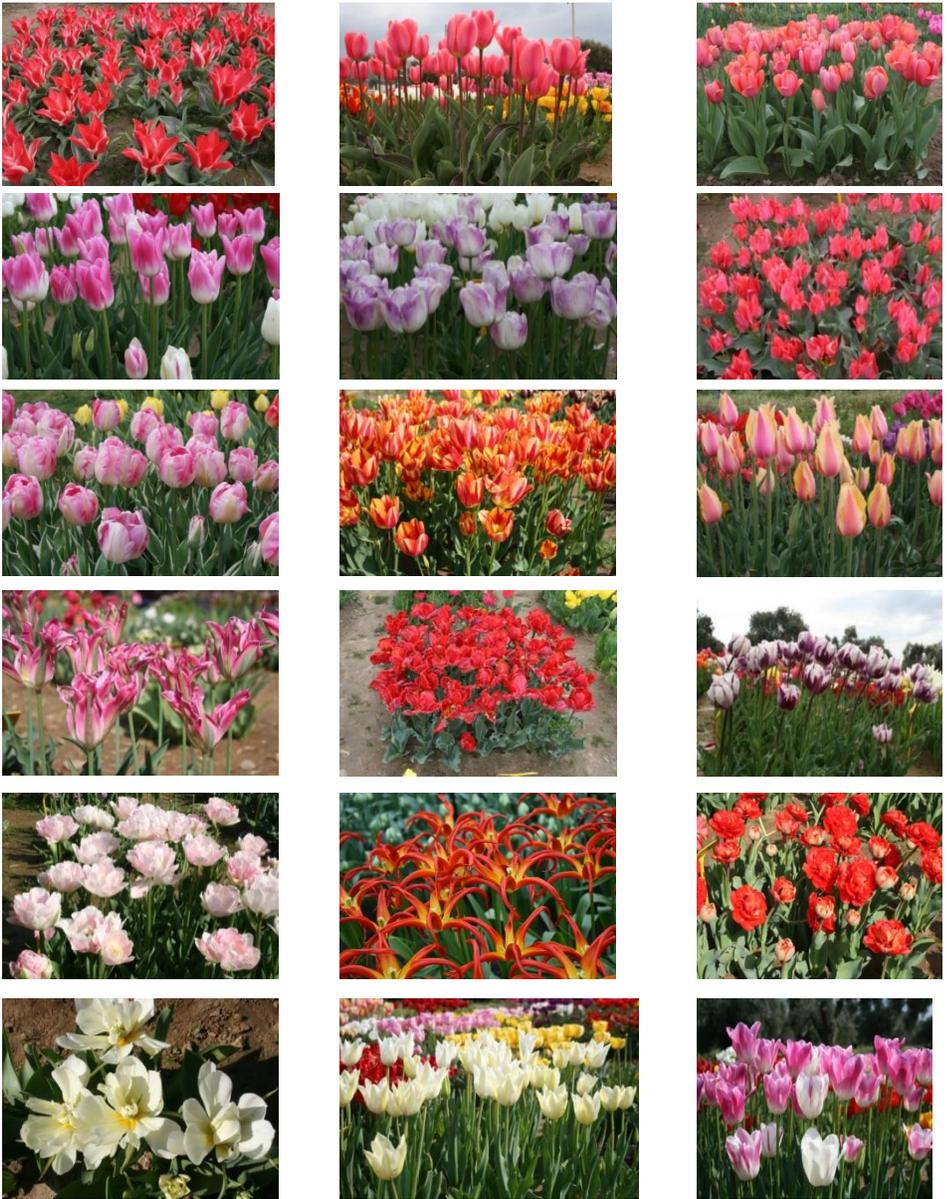


**Figure 5:** Tulip Seeds in Capsule (Original by Salman)

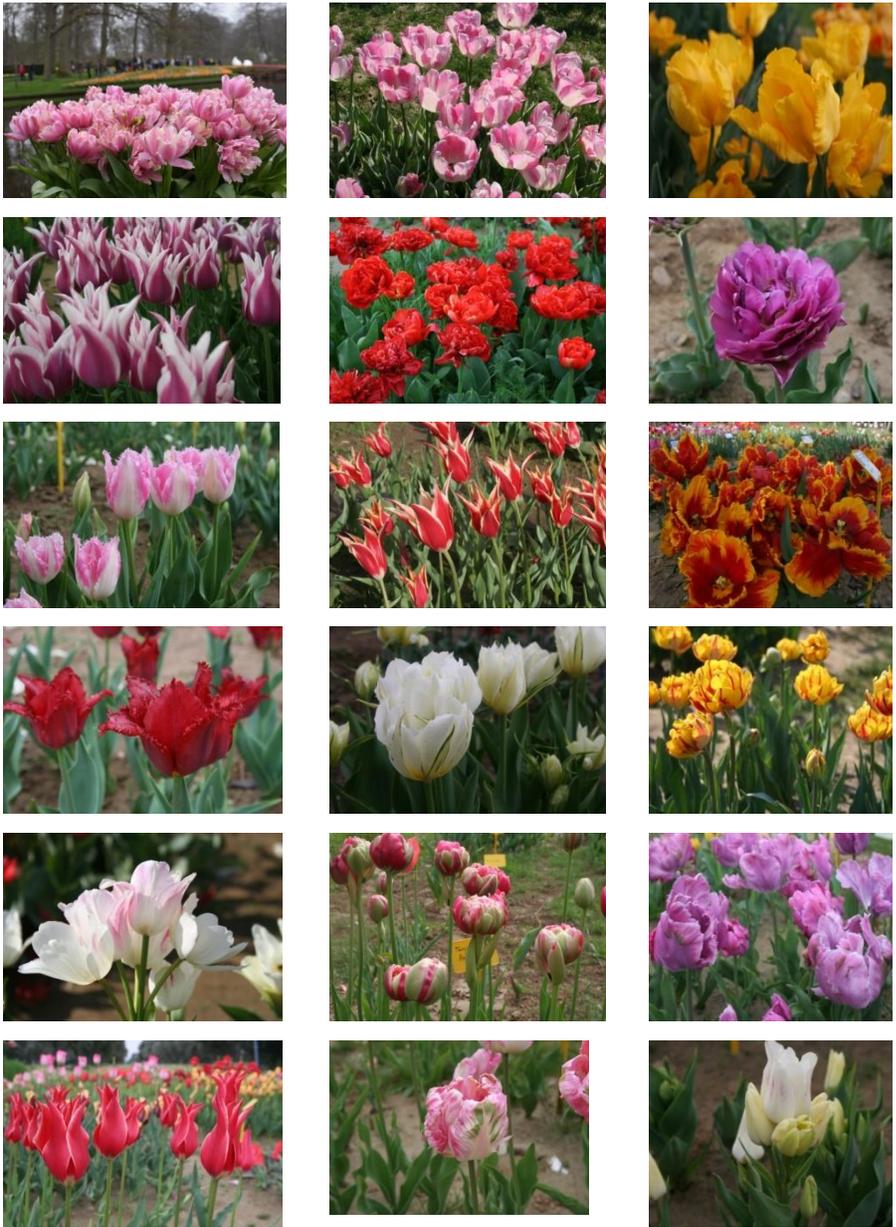


**Figure 6:** Seed Germination and First Bulblets (Original by Salman)

The native tulips in Turkey include 17 species, 4 subspecies and 3 varieties of total 20 taxa (Alp, 2021). The most notable among them are *Tulipa gesneriana*, *T. sprengeri*, *T. armena*, *T. cypria*, *T. slyvestris*, *T. julia*, *T. sintenesii*, *T. armena var. armena*, *T. armena lycila*, *T. sactatixatilis*, *T. pyracox*, *T. aganensis* and *T. orphaanidae*.



**Figure 7:** A Range of Tulip Cultivars - 1, Bayindir / Izmir (Original by Salman)



**Figure 8:** A Range of Tulip Cultivars – 2, Bayindir / Izmir (Original by Salman)

In Turkey, tulips are comprehensively propagated in Konya and Karaman in thousands of square metres. Annual bulb production changes according to demand which ranges between 20 and 100 million bulbs and is mostly directed to the domestic market. Also, in 2008 Istanbul Greater Municipality launched a social responsibility project (Tulip Bulb Cultivation Project with Purchase Guarantee) supporting the bulb production of local farmers in Silivri. Additionally, by organising an International Tulip Festival the municipality raised public awareness of the plant and the culture and heritage that lies behind it (Figure 9). As an overall result, the usage of tulip plants in landscape projects, especially those of the municipalities in Turkey, has shown notable increases, it has not yet reached the desired level. Nevertheless, it appears that the plant will be more sought after in the near future.



**Figure 9:** The International Tulip Festival in Istanbul (Original by Salman)

### **3. BULB PRODUCTION IN BAYINDIR – IZMIR**

Simultaneous research carried out between 2012-2014 in Istanbul, Izmir and Van focused on planting time and the regional adaptation of various cultivars to determine which of the comparable tulips were superior.

Bulb production of the tulip starts with soil preparation. The earth is prepared in autumn down to a level of 30 cm as the roots of the plant sufficiently grow down to 20 centimetres. The ground water must be kept at a depth of 50 to 60 centimetres and if necessary, a drainage system should be built accordingly. The physical and chemical aspects of the soil analysis must be executed at the same time to determine the required fertilizing schedule. The desired soil type is sandy loam as the moisture held by a heavy soil structure would damage the tulip bulbs over a prolonged period.

In warm regions it is particularly important to establish the planting time. It is determined that in Bayindir - Izmir, where the Mediterranean climate is dominant, the ideal planting time is mid-November. Earlier planting results in an occurrence of diseases and later planting prevents the plant from developing fully with a shorter vegetation period and blooming time. As the heat sets in rapidly in early spring this leads to lower bulb production.

In large fields the planting is carried out with machinery. On average 80 to 120 thousand bulbs could be planted in accord with the planting density.

Tulip bulbs are classified as <6, 6-8, 8-10, 10-12 and 12+ centimetres according to their perimeter. In bulb production an average 6 to 8 cm perimeter is accepted and those which fall below this value are dismissed. Suitable bulbs should be free of pests and diseases and have an intact and healthy tunic. Extremely dry, wounded, mouldy or rotten bulbs must be discarded.

Tulip bulbs can be planted directly into the soil with machinery (Figure 10) or in rainy climates in between nets. The planting should be carried out at a depth of 13 to 15 cm, 4 weeks before the first frosts. Bulbs must be irrigated immediately after planting is complete (Alp, 2021).

The tulip shoots occur in December or in January depending on the genetics of the cultivar (Salman et al., 2015). After the first shoots appear on the soil surface nitrate fertilizer (10-13 kg N/da) should be applied. The leaves must be kept clean of granules and irrigation should be carried out thoroughly to avoid fertilizer burn. The flowering period starts at the end of February and the beginning of March (Figure 11). Before the flowering time Calcium fertilizer (20 kg/da  $\text{CaNO}_3$ ) should be applied.



**Figure 10:** Bulb Planting with Machinery (Original by Salman)



**Figure 11:** Flowering Period on Tulip Field (Original by Salman)

Stem growth is continuous during flowering therefore; deadheading must be executed to increase the bulb yield (Figure 12 and Figure 13). In Turkey, this process is carried out by hand compared to the machine use in well developed countries.



**Figure 12:** Dead Heading by Hand (Left) and Machine Use (Right) (Original by Salman)



**Figure 13:** The Effects of Deadheading on Bulb Development (Original by Salman)

Fungi is the main cause of infections in Tulips. Additionally, some viruses and bacteria may affect the plant. Root rot (*Pythium spp.*), for example, is a soil born pathogen and spreads rapidly above 16 °C. The bulbs become soft and runny. To prevent root rot, the bulbs and soil

must be disinfected. Tulip grey bulb rot (*Rhizoctonia tuliparum*) affects the bulbs and leaves. Tulip fire or Botrytis blight (*Botrytis tulipae*, *B. cinerea*) creates an ash-coloured layer and can survive for years in soil therefore, bulbs and soil must both be sterilized before planting. *Penicillium stoloniferum* produces a layer of white mycelium on the bulbs, which turns into blue over the time. *Fusarium oxysporum* f. sp. *tulipae* causes withering on the tips of leaves and flower buds. In situations where *Sclerotium tuliparium* and *Sclerotinia bulborum* - which prefer damp and acidic environments - are detected tulip planting should be avoided for five years.

The main pests affecting tulip plants are nematodes, the European mole cricket (*Gryllotalpa gryllotalpa*), bulb mite (*Rhizoglyphus echinopus*) and aphids (Sarac et al., 2021; Anonymous, 2021b).

After the deadheading, to increase the bulb yield, Potassium nitrate ( $\text{KNO}_3$ ) should be applied 20 kg per decare. Irrigation is unnecessary for tulip growth until the soil begins to dry towards the end of spring. The leaves turn yellow at this time of the year which is also an indication of harvest time (Figure 14 and Figure 15). The harvest period takes place between May and June in warm climates, and in July in cooler regions. Harvested bulbs are left to dry in simple storage facilities at 30° C for a week before being cleaned and classified with further storage at 18-20 °C for up to a period of two months.

The cross section of newly harvested bulbs shows that the flower is not developed yet. Harvesting bulbs initiates the development of the flower

shoot inside the bulb (G stage) and this process is completed during the storage time (Figure 16).



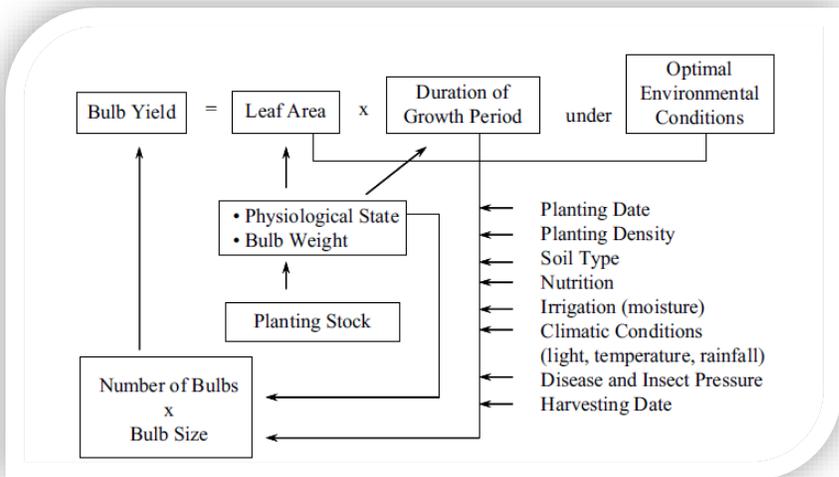
**Figure 14:** Tulips Ready for Harvest (Original by Salman)



**Figure 15:** Harvesting Tulip Bulbs with Machinery (Original by Salman)



**Figure 16:** Unexpanded Flowering Shoot (Original by Salman)



**Figure 17:** Schematic Relationships of the Major Factors Affecting Plant Growth and Bulb Production of Ornamental Geophytes (Le Nard & De Hertogh 2002)

There are several factors which affect the tulip bulb yield (Figure 17). These are environmental conditions including climate and soil type, planting time and density, growth period, leaf area of the chosen cultivar, nutrition, irrigation, insects and diseases, and harvest time (Le Nard & De Hertogh, 2002).

## **CONCLUSION**

Turkey, due to its geographical position, is suitable for production of many ornamental plants. In Turkey, tulip species are primarily used in landscape design both outdoors and for indoor use in pots which contrasts with their primary use in floriculture. The Netherlands, as the leading tulip producing country in the world collaborates with other Mediterranean countries (Spain, France, Italy) for early tulip propagation. Similarly, this research highlights the possibility of tulip production for floriculture being increased in the warmer regions of Turkey using the most adaptable cultivars of the plant. Therefore, the Tulip culture in Anatolia could be revived, domestic cultivars could be developed and the dependency in import could be reduced through related work.

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## **CHAPTER IV**

### **CUMULATIVE BIOCLIMATIC INDICES AND CLIMATE DATA OF RECENT YEARS IN SOME VITICULTURAL REGIONS OF TURKEY**

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## 1. INTRODUCTION

Turkey, a large peninsula surrounded by the Mediterranean Sea, the Black Sea, and the Aegean Sea, is considered one of the important countries suitable for viticulture regarding its location, climate, and gene resources. According to International Organization of Vine and Wine (OIV) data, it is estimated that the vineyard area worldwide was approximately 7.449 million hectares in 2018. In the same year, Turkey covered 6% of the world's vineyard area, following Spain (13%), China (12%), France (11%), and Italy (9%). Thus, these five countries represent nearly half of the global vineyard area (OIV, 2019; Santos et al., 2020). The production of grapes amounted to 77.137.016 tons globally and 4.100.000 tons in Turkey in 2019 (FAO, 2021). Ecological conditions allow diverse grape varieties to be grown in almost all parts of the country (Çelik et al., 1998). As a result, Turkey is considered one of the world's leading countries with more than 1200 grape varieties, most of which belong to *Vitis vinifera* L. species (Uzun & Bayır 2008; Söylemezoğlu et al., 2016).

Grape production in Turkey is comprised of table grapes (50% of all grapes), raisins (39%), and wine grapes (11%). The three top grape-producing locations ranked as Manisa (35.6%), Mersin (9.0%), and Denizli (8.8%), with Manisa being the most important raisin producing and exporting province in Turkey (TURKSTAT, 2020).

Grapes are produced intensively in the Aegean, the Mediterranean, and the Southeast Anatolian regions of Turkey. In addition, the

Mediterranean and south of the Aegean region are crucial for early-ripening grape cultivation. Moreover, plateau viticulture is also carried out in the Mediterranean region due to its higher mean temperatures compared to the other regions. The Thrace part of the Marmara region mostly comes to the forefront with the cultivation of wine grapes. Table grape varieties generally reach ripening in the mid and late seasons (Candar et al., 2021).

The amount of rainfall (over 2000 mm) seen at high altitudes in the Northeast Anatolia and the Black Sea regions limits grapevine growing (Çelik et al., 2008). Tokat province in the Black Sea region has a different situation with its particular geographical condition; viticulture is more developed than the other provinces in the same region. Particularly in the Black Sea region, grape cultivars (*Vitis vinifera* L.) may have some problems with fungal diseases, poor quality in berries, and generating sour grapes due to heavy rainfall in spring also autumn and insufficient sunshine during the vegetation period. However, it is known that grape varieties of *Vitis labrusca* L., which has a foxy flavor and is known to be resistant to fungal diseases such as powdery and downy mildew (Wan et al., 2007), are successfully grown in this region (Köse, 2014).

Sustainable viticultural practices in a region are strongly correlated to climate; environmental conditions of the vineyard areas affect grapevine growth, fruit ripening, and berry quality (Martínez-Lüscher et al., 2016; Rodrigues et al., 2021). Therefore, the suitability of a region for viticulture, the growth capacity of vines, and the capability

of grape ripening are usually determined by the climatic status of a region may predict with the help of different thermal indices such as the Winkler Index (WI) (growing degree days - GDD) and Huglin Index (HI). WI calculates the daily average temperature above a base set value of 10°C in the growing season of grapevines (from April to October for Northern Hemisphere) (Winkler et al., 1974). HI considers daily average temperature and daily maximum temperature, focusing on day length (Huglin, 1978; Jones et al., 2010).

Temperatures can vary each year to a small or a large extent. In addition, local factors (altitude, distance from the sea) may cause temperature variations. Nevertheless, this spatial variability in temperature often provides the most favorable factors for growing grapevines and its distinctive characteristics to a grape-growing terroir (Quénol, 2017; Morin et al., 2021). Viticultural regions in the same geography may have climatic differences according to their geographical location and latitude status. Because of the local factors and the distance from sea level, these differences can also be seen in different districts of the same province.

This chapter aims (1) to provide information about the situation of some viticultural regions of Turkey, (2) to present recent short-term (2015-2020) climate data of districts as Yunusemre (Manisa), Güney (Denizli), Şarköy (Tekirdağ), Kalecik (Ankara) and Erbaa (Tokat) and (3) to determine the WI and HI index values of districts by using short-term climate data.

## **2. MATERIAL AND METHODS**

### **2.1. Some Viticultural Regions In Turkey**

#### **2.1.1. Manisa**

Manisa province is located in the Aegean Region, western Turkey. It is bordered by Demirci, Bozdağlar, and Spil mountains in the north, south, northeast, and west; plains and plateaus of Gediz Basin area in the east. The climate of the province is a mix of continental and Mediterranean climates. While the Mediterranean climate is dominant in the plains and valleys in the province's lower altitude (A), the elevated parts of the province are influenced by the Central Anatolian continental climate (Koday et al., 2016). The altitude ranges between 43-850 m. Manisa is a significant province in Turkey in terms of grape production and vineyard area. According to 2020 data, 1.498.287 tons of grapes were produced in the 83.385 ha vineyard area (TURKSTAT, 2020). Apart from Yunusemre (A: ~80 m) and Şehzadeler districts located in the city center, Saruhanlı (A: ~43 m), Turgutlu (A: ~55 m), Ahmetli (A: ~86 m), Salihli (A: ~105 m), Alaşehir (A: ~189 m), and Sarıgöl (A: ~215 m) are the other districts in Manisa where grape production is intense. The most preferred grape variety by growers, Sultani Çekirdeksiz (Sultana), comprises almost all grape production in the region. Climatic characteristics of Manisa province enable the cultivation of numerous grape varieties, such as Bornova Misketi, Crimson Seedless, Antep Karası, Alphonse Lavallee, Michele Palieri, Trakya İlkeren, Red Globe, Mevlana, Superior Seedless, Razakı, Cabernet Sauvignon, Merlot, Syrah.

### **2.1.2. Denizli**

Denizli province is located in the inner Aegean Region in Turkey. The altitude of the city center is 354 m. There are many formations of mountains and plains (Yilmaz et al., 2020). The climate in the Aegean region is not fully effective in Denizli because of its distant location from the Aegean coastline, and the effects of the continental climate are common in the province. The mountains stretch perpendicular to the sea, and the province is usually open to the prevailing winds from the seas. Denizli ranks third in Turkey in terms of both vineyard area (35.677 ha) and grape production (371.603 tons) (TURKSTAT, 2020). Districts, where grape production is intense in Denizli, can be listed as Bekilli (A: ~850 m), Çal (A: ~850 m), Buldan (A: ~690 m), and Güney (A: ~850 m). While wine grapes such as Çal Karası, Öküzgözü, Syrah, Merlot, Cabernet Sauvignon, Kalecik Karası are grown more intensively in Güney and Çal districts, table grape varieties like Sultani Çekirdeksiz, Razakı, Mevlana, Superior Seedless are mostly grown in Bekilli and Buldan districts.

### **2.1.3. Tekirdağ**

Tekirdağ province, which is known as one of the suitability of wine grape cultivation, is located on the northwest coast of the Marmara Sea, reaching the Ergene and Istranca parts of the Marmara region. The vegetation of the province is characterized partly by forest and partly by shrubs. The eastern foothills of the Istranca Mountains stretch along the northeast of Tekirdağ (Özyavuz et al., 2018). Mediterranean climate effects are sensible on the coast of the Marmara Sea. However, snowfall

can be seen in the same region in winter. In Tekirdağ, 37.703 tons of grapes were produced in the 3.550 ha vineyard area (TURKSTAT, 2020). The route of Şarköy, Mürefte, Hoşkøy, Gaziköy, Uçmakdere, and Yeniköy is one of the most prominent regions of our country in terms of wine grape production. Whereas Semillon, Cinsault, Yapıncak, Gamay, Merlot, Papazkarası, Karalahna, and Adakarası grape varieties are cultivated for wine production (Bahar et al., 2010), Alphonse Lavallee, Cardinal, Michele Palieri, Italia, Yalova İncisi are generally grown as table grapes in these locations (Korkutal et al., 2018).

#### **2.1.4. Ankara**

Ankara is located in the center of Anatolia on the plains shaped by the Kızılırmak and Sakarya rivers. The elevation of the province is nearly 890 meters. The plains consist of folds and collapse zones enclosed by mountains and hills, which run from the southwest to the northeast (Arslan & Zeybek, 2019). Different climatic conditions are experienced in Ankara due to its wide geography. The steppe climate, the distinctive feature of the Central Anatolian climate, can be seen in the south. However, it is under the effect of Black Sea climate in the north, temperate and rainy. In this region, where the continental climate prevails, winter temperatures are relatively low, but the summer months are hot. The total grape production of Ankara is 28.770 tons in a 4.131 ha vineyard area (TURKSTAT, 2020). In terms of the vineyard area, the primary location is Kalecik (A: ~725 m), a town 70 km northeast of Ankara. Kalecik Karası is the most well-known grape variety in the region. Apart from Kalecik Karası, wine grapes such as Emir,

Hasandede, Narince, Papazkarası are mainly grown, while Alphonse Lavellee, Çavuş, Hamburg Misketi, Italia, Razakı, Kadın Parmağı, Gül Üzümlü varieties are produced as table grapes (Ağaoğlu et al., 2001; Çelik, 2011).

### **2.1.5. Tokat**

Tokat is located at the crossing zone between the middle Black Sea and the inner Central Anatolian regions. The general topography of the region shows mountainous character and different altitudes ranging between 85 and 2416 m. In addition, the region has several plains such as Niksar, Erbaa, Kazova alongside the river of Yeşilirmak and Kelkit stream, which are the well-known drainages (Dogan, 2007). Climate has a transition feature between the Black Sea and the step climate in Central Anatolia. In Tokat, the summer is generally warm and dry, while the winter could be very cold, partly cloudy, and snowy. Distance and elevation from the sea are the factors affecting the climate of Tokat. As a result, there are significant differences in the climate from North to South (Erdem, 2015). In Tokat, 58.937 tons of grapes were produced in 6.410 ha vineyard area (TURKSTAT, 2020). The main vineyard areas of the Tokat region are Central (A: ~625 m), Niksar (A: ~350 m), Turhal (Kazova Region) (A: ~500 m) and Erbaa (A: ~248 m) (Cangi et al. 2008; Bekar and Cangi 2017). The most grown grape variety is Narince. Although Narince is mainly known and cultivated as a wine grape in Turkey, it is also widely used for brined leaves production in Tokat.

## 2.2. Climatic Data of Districts

In the present study, it was considered that the temperature and precipitation data from five climatic stations (iMETOS IMT300, Pessl Inst.) located in various viticultural regions as the districts of Yunusemre (Manisa; A: 44 m), Güney (Denizli; A: 770 m), Şarköy (Tekirdağ; A: 46 m), Kalecik (Ankara; A: 706 m) and Erbaa (Tokat; A: 245 m) with climate data for the period between the years of 2015–2020. Furthermore, short-term climate data as daily maximum (Tmax), minimum (Tmin), and mean (Tmean) temperatures, total precipitation (mean data of years) were calculated. Location information of the climatic stations in districts is shown in Figure 1.



**Figure 1:** Location and Altitude Information of Climatic Stations

### 2.2.1. Winkler Index (WI) [Growing degree days (GDD)]

Winkler index was calculated with the sum of total degrees of mean daily temperatures above 10°C for each day during the vegetation

period. It is the most critical climatic criterion to determine the suitable grape variety for any region (Winkler et al., 1974). All calculations were made cumulatively with the formula in Table 1.

Some phenological observations and studies indicate that the vegetation period can start on March 1 to harvest time almost the end of September for some varieties in Manisa (Table 2). Therefore, an extra calculation was made with the data obtained from the Yunusemre climate station, and the months of the beginning of March and the end of September were also considered "Modified WI" for the Manisa region in this study.

**Table 1:** Winkler Index, Classification of Regions, Formula (Winkler et al., 1974; Carbonneau et al., 2007; Bahar et al., 2010; Candar et al., 2019)

<b>Winkler Index (WI – GDD)</b>	<b>Formula</b>
<b>Region I</b> (cold) < 1371	$\sum (T_{mean} - 10^{\circ}\text{C})$ <p>*<b>Tmean:</b> Daily mean temperature calculated for April 1 to October 31.</p> <p>*For Yunusemre climate station; data were also calculated between March 1 and September 30. This data was defined as "Modified WI" for the Manisa region.</p>
<b>Region II</b> (moderately cold) 1371 – 1649	
<b>Region III</b> (warm) 1650 - 1926	
<b>Region IV</b> (moderately warm) 1927 - 2205	
<b>Region V</b> (hot) > 2205	

**Table 2:** Phenological Chart for Some Varieties In Previous Studies in Yunusemre District

Varieties	Budburst (*EL 4)	Full Bloom (EL 23)	Harvest (EL 38)	References
Pembe Gemre	-	-	Sep. 25, 2002	(Ateş, 2007)
Trakya Ilkeren	Mar. 1, 2015	May 14, 2015	Jul. 12, 2015	(Yıldız & Dilli, 2018)
Cardinal	Mar. 1, 2015	May 20, 2015	Jul. 22, 2015	
Sultan 7	Mar. 7, 2015 Mar. 16, 2016 Mar. 20, 2017	May 6, 2015 May 10, 2016 May 16, 2017	Aug. 20, 2015 Aug. 25, 2016 Aug. 24, 2017	(Teket et al., 2018) (Teket & Altındışli, 2021)
Crimson Seedless	Mar. 15, 2017	May 17, 2017	Sep. 29, 2017	(Soltekin, 2019)

\*EL: Eichhorn-Lorenz system explains the phenological growth stage of grapevines (Coombe, 1995)

### 2.2.2. Huglin Index (HI)

Huglin Index (HI) is a variation of the GDD. It refers to a similar heat summation formulation based on an adjustment of maximum temperatures multiplied by a correction coefficient (k). In addition, it considers the mean daylight duration for the latitude where the studies are carried out (Table 3) (Jones et al., 2010). Same as the "modified WI" calculation, the Huglin index was also calculated for the Yunusemre district as named "Modified HI," and temperature data from the beginning of March to the end of September were used.

**Table 3:** Hugin Index, Classification of Climates and Formula (Huglin, 1978; Tonietto & Carbonneau, 2004)

Huglin Index (HI)	Formula
<i>Very cool</i> (HI-3) < 1500	$\sum ((T_{mean} - 10^{\circ}\text{C}) + (T_{max} - 10^{\circ}\text{C})/2) * k$ <p><b>Tmean:</b> Daily mean temperatures calculated for April 1 to September 30.</p> <p><b>Tmax:</b> Daily maximum temperatures calculated for April 1 to September 30.</p> <p><b>k:</b> Length of the day coefficient was calculated as;                      1.00 for <math>\leq 40^{\circ}00'</math> latitude (Yunusemre and Güney districts)                      1.02 for <math>40^{\circ}.01' - 42^{\circ}.00'</math> latitude (Şarköy, Kalecik and Erbaa districts)</p> <p>*For Yunusemre climate station, data were also calculated between March 1 and September 30. This data was defined as "Modified HI" for Manisa region.</p>
<i>Cool</i> (HI-2) 1500 - 1800	
<i>Temperate</i> (HI-1) 1800 - 2100	
<i>Temperate warm</i> (HI+1) 2100 - 2400	
<i>Warm</i> (HI+2) 2400 - 2700	
<i>Very warm</i> (HI+3) > 2700	

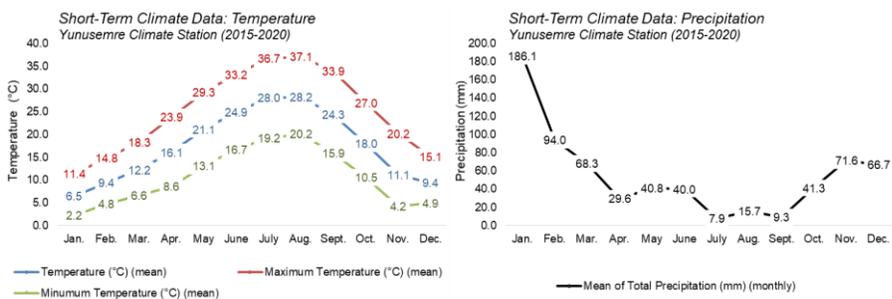
### 3. RESULTS AND DISCUSSION

#### 3.1. Climatic Characteristic of Districts (2015-2020)

The macroclimate of Yunusemre district (city center of Manisa) can be classified as mild, hot and dry summers, as the general characteristics of the Mediterranean climate. Mean values of climate data between 2015 and 2020 show that Yunusemre had high values with a maximum (36.7 °C and 37.1 °C) and mean (28.0 °C and 28.2 °C) temperatures in July and August. The mean annual temperature of the years was found as 17.4 °C. Although the mean of total precipitation amounts of years was 671.3 mm in this region, it is notable that the precipitation in the summer period is deficient (Figure 2). Especially in July (7.9 mm) and

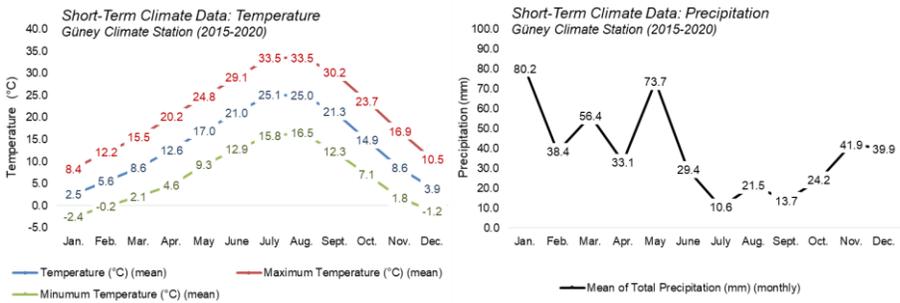
August (15.7 mm), irrigation applications in the region are generally necessary due to insufficient precipitation. The long-term mean annual temperature between 1930-2020 was 16.9 °C; the mean temperature of the growing season (April to October) was 22.5 °C; the mean maximum temperature for June, July, and August was found as 33.9 °C. Between these years, annual precipitation, precipitation in the growing season, and the total amount in July and August were determined as 744.4 mm, 205.7 mm, and 19.2 mm, respectively (TSMS, 2021).

The mean values of the short-term climate of Güney, which is another district located in the Aegean Region, showed that the mean and maximum temperatures were 19.6 °C and 27.9 °C, respectively, in growing seasons (April-October). With a mean temperature of 25.1 °C, July came to the fore as the warmest month in these years. The climate of the Güney district is generally characterized as warm during the day and cool at night. For this reason, the region has recently become a hotspot for wine grape cultivation.



**Figure 2:** Average Climatic Data Of Yunusemre Station (2015-2020)

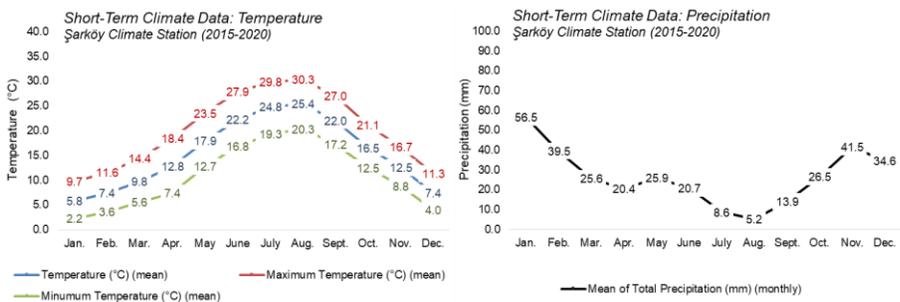
While 206.2 mm precipitation (44.5% of the mean annual total) was recorded from April to October, almost dry conditions were experienced from June to August, with total summer precipitation (61.5 mm) less than 86.7% of the annual value (Figure 3). Denizli's long-term climate data (1957-2020) indicated that the mean annual temperature was 16.2 °C and total annual precipitation was 569.2 mm. Precipitation was well balanced all along the year but slightly lower in the summer season (TSMS, 2021). On the other hand, Güney also has a more continental climate and is cooler than the center of Denizli due to its higher altitude (A: ~800 m). The fact that the annual mean temperature was determined as 13.8 °C in the short-term climate data of the study supports this situation.



**Figure 3:** Average Climatic Data of Güney Station (2015-2020)

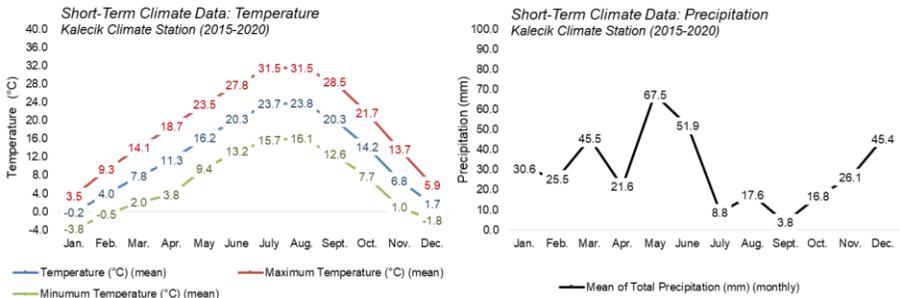
Şarköy district, which is considered an essential region for cultivating many wine grape varieties, is generally located on the south-facing slopes of the Ganos Mountains and in the east and northeast directions of the inner valleys. The vineyard areas in the region can be in different altitudes between 650-700 meters from sea level (Bahar et al., 2010).

This study used the climate records from the Şarköy station located at an altitude of approximately 46 m. The mean values of short-term climate data recorded for the annual temperature were 15.4 °C, for the mean maximum temperatures were 29.8 °C and 30.3 °C in July and August, respectively. While the mean temperature of the growing season (April-October) was 20.2 °C, the maximum temperature was 5.2 °C higher than the mean value (Figure 4). The Şarköy district in Tekirdağ province's experiences in short-term climate data show that the annual precipitation value of six years was 319.1 mm. 10.8% of the mean annual precipitation of six years was between June and August, 121.3 mm of precipitation during the growing season (April-October). The long-term mean annual temperature determined between 1939-2020 in Tekirdağ was 14.1 °C; the mean temperature of the growing season (April to October) was 19 °C; the mean maximum temperature between June and August was 27.2 °C. While the annual mean precipitation in long-term years was 583.1 mm, the average total precipitation in the summer (June to August) was 78.0 mm (TSMS, 2021).



**Figure 4:** Average Climatic Data of Şarköy Station (2015-2020)

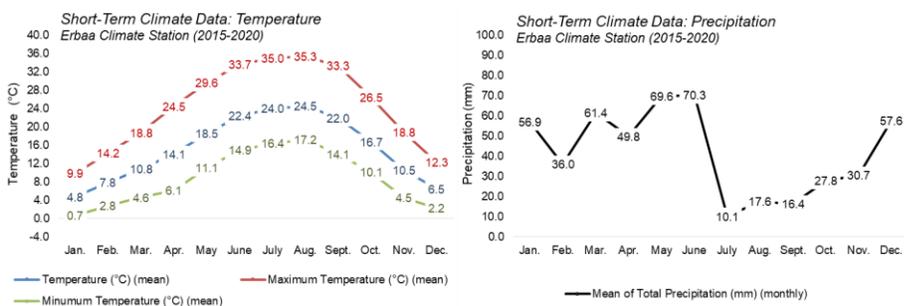
Kalecik district had the lowest annual mean temperature (12.4 °C) compared to other regions in this study due to its topographic and geographical situation. The mean temperature was 19.7 °C in the growing season (April to October) and 22.6 °C in the summer months (June to August). The highest mean maximum temperature was 31.5 °C. The total annual precipitation mean of years was determined as 361.2 mm. It has been determined that 52.0% of the average annual precipitation value occurs during the growing season, and 21.7% is only in the summer months (Figure 5). According to the long-term data (1927-2020) in Kalecik district, mean annual temperature, mean temperature in the growing season (April to October), and mean temperature in the summer (June to August) were found as 11.9 °C, 18.0 °C, and 22.3 °C, respectively. In addition, it is indicated that 51.5% of the total precipitation (393.2 mm) occurred in the growing season and 15.7% in the summer months. (TSMS,2021).



**Figure 5:** Average Climatic Data of Kalecik Station (2015-2020)

Short-term mean temperature data recorded in the Erbaa district has shown similarities to the Yunusemre district presented in the study.

According to six years average in the Erbaa district, the annual mean temperature was 15.2 °C; the mean temperature in the growing season (April to October) was 20.3 °C; the mean maximum temperature for the hottest month (August) was 35.3 °C. Furthermore, while annual precipitation was 504.2 mm, precipitation for the growing season was 142.1 mm. It was observed that there was a sharp decrease in the six-year average of precipitation from June to July (Figure 6). 27.7 mm precipitation occurred in only July and August on average over the years. The long-term mean annual temperature determined for the years between 1929-2020 was 12.4 °C; the mean temperature of the growing season (April to October) was 17.9 °C; the mean maximum temperature between June and August was 29.5 °C. While the annual mean precipitation in the long term was 435.2 mm, the average total precipitation in the summer months (June to August) was 20.2 mm (TSMS, 2021). Erbaa (A: ~248 m). differs based on climate compared to the center of Tokat (A: ~625 m) because it is located at a lower altitude.



**Figure 6:** Average Climatic Data of Erbaa Station (2015-2020)

### **3.2. Winkler Index (WI) (Growing degree days – GDD) Values**

The Winkler Index (WI) categorizes regions that accumulate heat summation units by adding hours above 10 °C during the growing season from April to October (Winkler et al., 1974). WI presents climatic regions using total GDD from Regions I (cold) to V (hot). Climates of these regions are defined in Table 4.

According to the WI classification, the GDD data averages between 2015-2020 were calculated as 2786.0 for Yunusemre (April-October) and 2619.2 for adjusted Yunusemre (March-September). These findings indicate that the Yunusemre district can be classified as a hot climate (GDD > 2205) in Region V according to the classification of WI. Yıldız & Dilli (2018) calculated the effective heat summations for some table grapes as 1206 GDD for Cardinal, 1223 GDD for Trakya İlkeren, 1381 GDD for Yalova İncisi and 1381 GDD for Italy under Yunusemre climatic conditions. These results showed that early-ripening varieties could reach ripening in the second week or at the end of July in the Yunusemre district. Moreover, many grapes varieties could have sufficient ripening during the harvest time of the Yunusemre district. In this regard, this research clearly showed that the WI values of the Yunusemre district are pointing that early-ripening varieties could easily reach maturity in July, mid-season varieties in late July, or mid-August late-ripening varieties in September.

The average of six years of GDD values for the Güney district was found as 2067.9. Remarkably, the total amounts of GDD obtained at

the end of September and October in Güney district may limit the maturing of late-ripening varieties. Therefore, the desired Brix amount may not be reached for Boğazkere, Öküzgözü, Crimson Seedless, Pembe Gemre, and similar late-ripening grape varieties due to insufficient pre-harvest temperatures. Moreover, in case of possible heavy rains before the harvest time (October) in this region, the emergence of fungal infections may adversely affect the grape quality.

Short-term climate data showed that the average value of Şarköy district was 2212.1 GDD. The Şarköy climatic station, which provided data in this study, is located at an altitude of 46 m, closer to the sea level. However, Bahar et al. (2010) reported that vineyards were spread over a wide area in Şarköy, and some vineyards can be located at an altitude of 650 - 700 m. Therefore, they determined the WI as 1959 GDD (Region IV). Candar et al. (2019) calculated WI over six years for Tekirdağ (Süleymanpaşa district, A: 4m) between the years of 2013 - 2018 and found that values ranged from 1968.0 (2017) to 2235.0 GDD (2018).

Kalecik district, which had the lowest average WI with a value of 1866.9 GDD in the present study, was classified as Region III (Table 4). Çelik et al. (2015) reported that Öküzgözü, Boğazkere, Yapıncak and Papazkarası grape varieties grown in the Kalecik district required a heat summation of 1783 GDD, 1787 GDD, 1821.0 GDD and 1835.0 GDD, respectively.

**Table 4:** Short-Term Cumulative GDD Values of Years (Mean values, 2015-2020)\*\*

Stations Months	*Yunusemre (MANİSA)	Yunusemre (MANİSA)	Güney (DENİZLİ)	Şarköy (TEKİRDAĞ)	Kalecik (ANKARA)	Erbaa (TOKAT)
Mar	81.6	-	-	-	-	-
Apr	265.1	183.5	94.6	91.8	72.6	127.6
May	609.5	527.9	312.1	338.1	265.4	397.2
Jun	1065.1	983.5	642.3	712.7	570.9	777.2
July	1624.1	1542.5	1110.5	1171.2	993.7	1222.1
Aug	2189.6	2108.0	1575.4	1649.4	1420.1	1683.7
Sep	2619.2	2537.6	1913.5	2009.8	1730.5	2042.3
Oct	-	2786.0	2067.9	2212.1	1866.9	2251.3

\*Adjusted data (March to September)

Classification of Regions Winkler Index (GDDs)	Region I Cold <1371	Region II Mod. Cold 1371-1649	Region III Warm 1650-1926	Region IV Mod. Warm 1927-2205	Region V Hot >2205
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\*\* Annual cumulative GDD values are shown in Figures 7,8,9,10,11 and 12

On the other hand, Kunter et al. (2017) determined the WI value of the Kalecik district as 2084.0 GDD, and the study showed that Öküzgözü (1922 GDD) and Boğazkere (1926.0 GDD) grape varieties required the highest total heat summation values compared to the other 36 varieties. Therefore, previous studies and the findings of this study have shown that the heat summation requirements above 1870 GDD of some varieties may cause problems such as insufficient ripening in Kalecik district.

The average value of the last six years obtained from the Erbaa climate station was 2251.3 GDD (Region V). Although the Erbaa district is located between the Black Sea and the Central Anatolia regions, it was classified as a warm region. Kılıç et al. (2018) reported that the heat

summation values of some table grape varieties were between 1008 and 1748 GDD, according to phenological studies conducted in between 2014-2015. The study showed that some table grape varieties could easily be adapted to the Erbaa district compared to the center of Tokat. With this, they reported that early-ripening varieties Trakya İlkeren and Prima, mid-season varieties Cardinal, Victoria, and Lival, late-ripening varieties Michelle Palieri and Italia could potentially be grown in Tokat Central. While that is the case, this study showed that many table grape varieties could be grown with the value of 1683.7 GDD for the end of August, with 2042.3 GDD for the end of September and with 2251.3 GDD for the end of October in the Erbaa district. However, monitoring yield and quality parameters and considering other climatic conditions such as precipitation and humidity are highly recommended.

Previous studies have shown that there were climatic differences between the Tokat Central and the Erbaa district. Bekar & Cangi (2017) reported that the heat summation values of the Narince grape variety were determined as 1885.9 GDD for the center of Tokat and 1842.9 GDD for the Erbaa district, according to the 2014 climatic data. Furthermore, Cangi et al. (2008) conducted a study to determine the heat summations for grape varieties in Kazova (Turhal) (A: 540m), another district of the Tokat province. In the study, while Çavuş (1583.3 GDD) and Cabernet Sauvignon (1629.4 GDD) were determined as the earliest ripening varieties, Boğazkere (1816. GDD) and Öküzgözü (1810.2) were the latest ripening varieties.

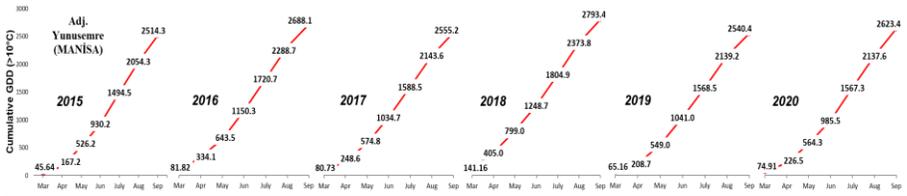


Figure 7: Cumulative GDD Values by Years (Adjusted Yunusemre District)



Figure 8: Cumulative GDD Values by Years (Yunusemre District)

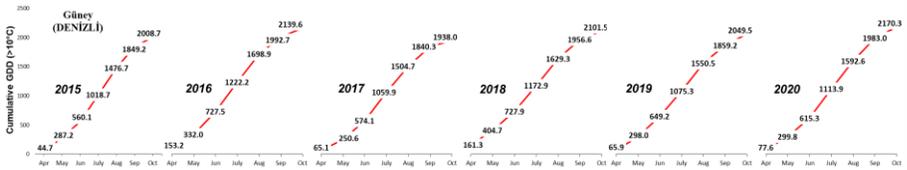
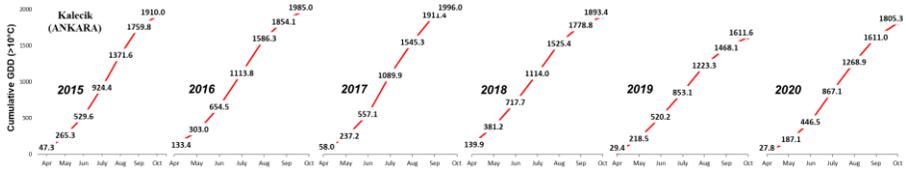


Figure 9: Cumulative GDD Values by Years (Güney District)



Figure 10: Cumulative GDD Values by Years (Şarköy District)



**Figure 11:** Cumulative GDD Values by Years (Kalecik District)



**Figure 12:** Cumulative GDD Values by Years (Erbaa District)

### 3.3. Huglin Index (HI) Values

Huglin Index (HI) is another type of bioclimatic heat index which calculates the heliothermic potential of viticultural regions. Although the calculation of daily average temperatures is similar to the Winkler index, the daily maximum temperature and the latitude information of the calculated location are also considered. It classifies from HI-3 to HI+3, which defines "very cool" to "very warm," as shown in Table 5. HI shows the correlation between the quality and the temperature for wine grape varieties in general terms. In some regions where the grapevine is cultivated, it is reported that these values should not be less than 1500 (Huglin 1978; Tonietto & Carbonneau, 2004) or 1600 (Laget et al., 2008).

The mean HI (2015-2020) values for Yunusemre (April-September) and 'adjusted Yunusemre' (March-September) were calculated as

3320.0 and 3483.9, respectively. Both calculations for the Yunusemre district showed that the climate was classified as HI+3 (very warm). Güney district was also classified as HI+2 (warm) climate for viticulture with 2654.2 value. Güney district, where the difference between day and night temperatures is high due to its location, the recorded daylight temperatures were quite different from night temperatures. Since the HI also focuses on the maximum temperatures, HI values differed from WI values for the Güney district.

The HI value of the Şarköy district was determined as 2535.4, and the climate was classified as "HI+2, warm". Bahar et al. (2010) also indicated the same climate class (2223.0) for the Şarköy district. Another study conducted by Candar et al. (2019) revealed that the six-year HI values for the Şarköy district were ranged from 2223.0 (2017) to 3044.7 (2013). In this study, the lowest HI value was found in the Kalecik district. Therefore, the district's climate defined "HI+I, moderately warm" with a value of 2386.1. Even though the Erbaa district is located in the Black Sea region in Turkey, the HI value was determined as "HI+3, very warm" like Yunusemre district due to its geographical location, altitude, and climatic conditions.

**Table 5:** Short-Term Cumulative Huglin Index Values of Years (Mean, 2015-2020)

Stations Months	*Yunusemre (MANİSA)	Yunusemre (MANİSA)	Güney (DENİZLİ)	Şarköy (TEKİRDAĞ)	Kalecik (ANKARA)	Erbaa (TOKAT)
Mar	163.8	-	-	-	-	-
Apr	462.3	298.4	197.1	172.6	155.2	264.7
May	933.5	769.7	534.5	511.7	464.1	716.9
Jun	1514.6	1350.7	986.1	978.4	891.1	1278.7
July	2207.8	2044.0	1584.4	1526.0	1388.5	1907.8
Aug	2911.1	2747.3	2181.8	2091.2	1945.1	2550.7
Sep	3483.9	3320.0	2654.2	2535.4	2386.1	3090.0

\*Adjusted data (March to September)

Huglin Index (HI)	HI-3 Very Cool <1500	HI-2 Cool 1500-1800	HI-3 Temperate 1800-2100	HI+1 Temperate Warm 2100-2400	HI+2 Warm 2400-2700	HI+3 Very Warm >2700
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Different evaluations regarding the determination of the Huglin Index class ranges have been reported. For example, while some researchers stated that the HI+2 ranking could be between 2400 and 3000, and the HI+3 ranking could be over 3000 (Tonietto & Carbonneau 2004), others reported that two more classes could be added like "too cool (<1200)" and "very cool (1200 - 1500)" (Honorio et al., 2018). However, considering the classifications in previous studies, it was understood that the findings in this study did not cause a problem, especially in terms of evaluation of "warm" and "very warm" classification values.

## CONCLUSION

Monitoring the climatic suitability of a location for grape growing is critical for the viticulture industry in the context of climate change. In

this study, Winkler and Huglin Index calculations were presented according to the temperature values recorded in recent years in some important viticultural regions such as Yunusemre (Manisa), Güney (Denizli), Şarköy (Tekirdağ), Kalecik (Ankara), and Erbaa (Tokat) districts in Turkey. Moreover, brief information about the climate data, geographical status of locations, and the varieties grown in these regions were provided.

Winkler Index regional classifications based on short-term climate data showed that Yunusemre, Şarköy, and Erbaa districts were located in Region V (hot), while Güney district was involved in Region IV (moderately warm). However, Kalecik district was included in the Region III (warm) classification. All these findings showed that when deciding on the selection of late-ripening varieties for Güney and Kalecik districts, it is necessary to overthink in detail and ensure the adaptation capability of the varieties for these regions. As for Huglin Index of locations, it was determined that Yunusemre and Erbaa districts were in the same classification (HI+3, very warm), while Güney and Şarköy districts are between 2400-2700 (HI+2, warm). Nevertheless, Kalecik district differentiated with the values between 2100 and 2400 as HI+1 (temperate warm) compared to other places in this study.

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## CHAPTER V

### GRAPE POMACE - A PROMISING BIOCOMPONENT AS BIOSTIMULANT AND ECO-FERTILIZER. A REVIEW

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## INTRODUCTION

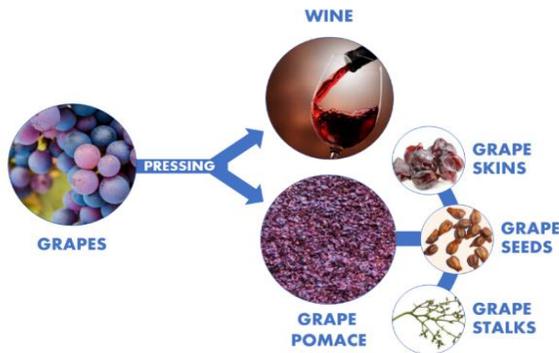
Grapevine represents one of the world's major crops. This perennial plant was first cultivated approximately 6000-8000 years ago, and as of 2019, it occupied 7.4 million hectares. In the same year, 260 millions of hectolitres of wine were produced, along with a great amount of residual grape skins, seeds and stems, their mixture being called "grape pomace" or "grape marc". Considering that for each 6 litres of wine results 1 kg of grape pomace, in 2019 only, 4.3 megatons of pomace were produced (OIV, 2020).

The considerable quantity of these solid remains from the vinification process creates a disposal issue, especially from the environmental and economic points of view. Scientists have been searching for ways of recycling and harnessing the potential of this by-product and have found applications in animal feed (Beres et al., 2016), as compost for soil amendments (Salgado et al., 2019), as a thermal insulator for buildings (Muñoz et al., 2014), to count a few. The potential of grape pomace can be also harnessed in the form of nutraceutical and pharmaceutical supplements, thanks to the high dietary fibre and polyphenol content, while the grape seed oil and antioxidant properties make grape pomace an important source of valuable cosmetic ingredients (Galanakis, 2017; Brezoiu et al., 2020; Abduraman et al., 2020). As a growth of interest has been noted for the valorization of this kind of winery residues, a large spectrum of applications has been developed, from fields that are very diverse.

This review summarizes different aspects of grape pomace applications in horticulture and agriculture as biostimulant-regenerator and ecological fertilizer.

## 1. OBTAINING GRAPE POMACE

During the pressing process, 2 kinds of products are obtained from grapes: the grape juice, known as must, which will eventually become wine, and the grape pomace (Figures 1a, 1b). In the case of white wine, the grape marc is discarded immediately, while for the red wines, pressing residues are left in the must for a certain time period, in order to make the extraction of grape constituents (such as polyphenols and pigments) possible. After the maceration time has ended, the pomace may be taken out of the must and discarded. Pectolytic enzymes or cellulases are usually added in the must extraction or maceration processes, with the aim of obtaining a greater yield or a more intense colour (Galanakis, 2017).



**Figure 1a:** By-Products Obtained From Grapes



**Figure 1b:** Grape Pomace Obtained at Research Station for Viticulture and Oenology Murfatlar, Constanta County, Romania (Original by Artem)

## **2. PHYSICAL-CHEMICAL PROPERTIES**

This important by-product represents approximately 15 to 25% of the total weight of the grapes, percentage varying in accordance with the wine-making process and the grape variety (Oliveira & Duarte, 2016). From a physico-chemical point of view, after pressing, the grape pomace has a pH in the range of 6.5 to 8.5, low electrical conductivity, a moisture level of 20-30% w/w, and a high organic matter content (García -Lomillo & Gonzalez-SanJose, 2017). Grape pomace is also rich in valuable products such as mineral substances, ethanol, dietary fiber, hydrocolloids, citric acid, tartrates, malates, and ethanol (Kosseva & Webb, 2020).

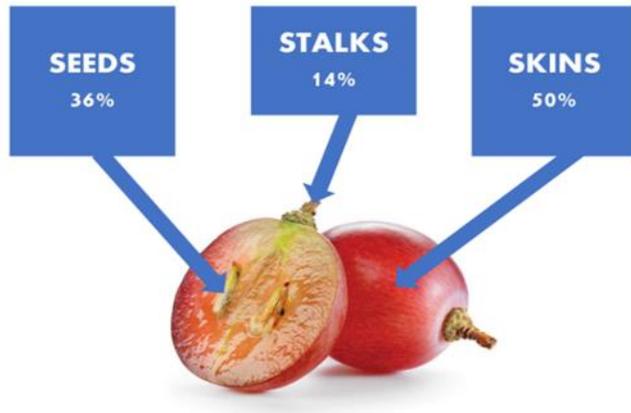
Grape pomace generally comprises 30% neutral polysaccharides, 20% polygalacturonic acid, 15% insoluble proanthocyanidins, 6-15% structural proteins, a wide diversity of phenols, lipids and lignin. The phenols and the structural proteins are an important source of organic compounds when they are obtained by fractioning, as they are bonded to lignin-carbohydrate complexes (Minjares-Fuentes et al., 2014).

In the case of red wine, the grape pomace is fermented, and in the end, it contains a very low amounts of sugars and phenols. The quantity of these constituents may vary as well, depending on the vinification processes and grape varieties. White grape pomace, on the other hand, is unfermented and has a greater quantity of carbohydrates and phenolic compounds, in comparison with the red, fermented pomace (Owen, 2015; Sirbu et al., 2021).

### **3. GRAPE POMACE COMPONENTS**

Grape pomace consists of roughly 50% skins, 36% seeds and 14% stalks (Figure 2). However, these proportions may vary, depending on the grape variety. Nonetheless, the skins are the predominant constituent, being represented by approximately one half of the grape pomace weight (Mendes et al., 2013).

Grape pomace has high quantities of phenolic antioxidants that make up 10 - 11% of the dry weight. Phenols are plant secondary metabolites, which, from a chemical point of view, contain a benzene ring with one or more hydroxyl groups. Polyphenols are organic compounds with more than one phenol units (Galanakis, 2017).



**Figure 2:** Approximate Composition of Grape Pomace (Galanakis, 2017)

Polyphenols found in foods are grouped into 4 major classes, namely stilbenes, flavonoids, phenolic acids, and lignans. The most commonly found stilbene of vegetal origin is resveratrol, a phytoalexin produced by plants as a response to being injured or attacked by pathogens, thus being a natural fungicide; flavonoids are the most intensely studied group of polyphenols, which comprises isoflavones, anthocyanidins, and proanthocyanidins; phenolic acids are represented by hydroxybenzoic and hydroxycinnamic acids, the latter including caffeic, chlorogenic, ferrulic, gallic, *p*-coumaric and sinapic acids. The polyphenol content of the grape pomace is variable, being determined by climate, location, grape maturity and the duration of the fermentation process (Pinelo et al., 2006).

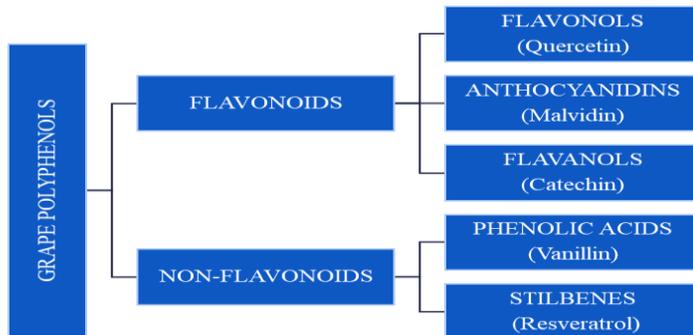
### 3.1. Grape Skins

The skins have a great amount of fibers and phenolic compounds, a protein content ranging from 5 to 12%, 2 - 8% ash and a soluble sugar

content in the range of 1 to 70%, the latter being conditioned by the applied vinification processes (Galanakis, 2017).

The main constituent of grape skins is dietary fiber, representing up to 60% of the dry matter, being almost entirely made up of the insoluble kind. According to Pinelo et al. (2006), the cell wall of the grape skins comprises neutral polysaccharides such as cellulose, arabinan, mannan, galactan, xylan and xyloglucan, substances of acidic pectin nature, lignin, structural proteins and proanthocyanidins.

Polyphenolic molecules are located in the highest amount in the skin of the grape berries, these compounds reaching a great diversity in this part of the fruit (Figure 3).



**Figure 3:** Types of Grape Polyphenols Found In the Grape Skin and Examples

The skin of red grapes contains flavonols, hydroxycinnamic acids and, according to Benmeziane et al., (2016), anthocyanin compounds such as acylated anthocyanins, coumaroyl-acetylated anthocyanins, coumaroylglucoside anthocyanins, cyanidin, delphinidin, malvidin, non-acylated anthocyanins, peonidin and petunidin. This grape skin anthocyanin profile is correlated with the intensity of the berry color

and although it is genetically determined for each grape variety, most of the times it is dependent on the agronomical and environmental factors. The skin of white and red grapes does not differ significantly, except for the fact that the skin of red grapes contains anthocyanins, whereas the skin of white grapes contains minor phenols like vitexin (Pinelo et al., 2006).

The applied winemaking techniques can also influence the polyphenol content of the grape skins from grape pomace. For red wines, the skins are left in the fermenting must for a certain period of time, which results in a polyphenolic extraction in the hydroethanolic solution that results from the fermentation process. Nonetheless, at the end of the maceration-fermentation step, the skin still contains a great amount of polyphenols, which can be used in various applications, due to their valuable properties (Galanakis, 2017).

### **3.2. Grape Seeds**

Grape seeds constitute between 2 - 5% of the fresh grape weight (Choi & Lee, 2010) and approximately 36% of the grape pomace resulted from the pressing process. However, these proportions may vary, as not all grape varieties have the same number and weight of seeds (Galanakis, 2017).

Their main constituent is fiber, representing 40% of the total mass. Grape seeds contain 10 - 20% lipids, 10% proteins, 5 - 8% complex phenolic compounds sugars, and minerals (Rockenbach et al., 2012). The fiber content is represented by 80% cellulose and pectin, the

percentage taking into account the dry matter, without the sugars (Kosseva & Webb, 2020).

One of the reasons for which grape seeds are valuable is the fact that they are rich in tocopherols and unsaturated fatty acids: linoleic acid constitutes 66.76 - 73.61%, oleic acid 17.8 - 26.5%, palmitic acid 6.35 - 7.93%, and stearic acid 3.64 - 5.26% (Owen, 2015). The use of grape seed oil has applications in the cosmetic, food, pharmaceutical and biodiesel industries (Górnaś & Rudzinska, 2016).

Polyphenolic compounds are another important component of grape seeds. The most notable are flavonoids, phenolic acids, resveratrol and procyanidins. The polyphenol composition of the grape seeds, like many other aspects related to the grape pomace, depends on a multitude of factors, such as the variety, the ripeness degree of the grapes, and also the soil and climate where the grapes were grown (Bordiga et al., 2015; Galanakis, 2017).

The tannins present there share the same component units as those present in the skin, but the polymerization degree of the seed tannins is lower, reaching a value of 11, because most of the times they are found in the monomeric form. The gallate levels, however, are 30% higher in the grape seeds, in comparison with the stalks and skins (Pinelo et al., 2006).

This portion of the grape berry has the highest antioxidant activity. Besides the polyphenolic compounds, the grape seeds also contain non-

phenolic antioxidants, such as carotenoids, vitamin E, and phytosterols, mainly present in the lipid components (Owen, 2015).

### **3.3. Grape Stalks**

Grape stalks are the skeleton of the grape cluster, constituting approximately 3 - 5% of the weight of the fresh grape and 14% of the grape pomace. The stalks contain lignocellulosic compounds (hemicelluloses and cellulose), between 22 and 47% lignin and are rich in tannins, which amount about 16% of the total mass (Galanakis, 2017). The latter represent approximately 80% of the phenolic compounds found in grape stalks, and their polymerization degree averages 9 (Souquet et al., 2000).

Tannins such as flavonols, catechin, and galocatechin are bonded with lignin, which has a higher condensation degree in comparison with conventional lignins from other agricultural residues and from wood, thus making it very difficult to extract pure cellulose from them (Prozil et al., 2014).

## **4. IMPACT ON THE ENVIRONMENT AND BIOECONOMY**

The viticulture and winemaking industry has to face a serious environmental problem: the adequate disposal of grape pomace (Patti et al., 2009). Scientists and vintners have tried to find sustainable methods for discarding wine residues, as the by-product quantity increases proportionally with the growth of the wine industry (Dobrei et al., 2016).

Due to its high phenolics content, grape pomace has a significant impact on the environment, as these compounds increase the biochemical and chemical oxygen demands (Owen, 2015). Soil degradation, water pollution, flora deterioration and odour emissions are examples of unwanted effects produced by wineries, through which the environment is affected. Thus, it is compulsory to find means of mitigating the scale of the consequences, through technologies which allow the recovery and reuse of vinification by-products (Oliveira & Duarte, 2016).

The impact is not only felt speaking from an environmental point of view, but also economically (Galanakis, 2017). Grape pomace has a high economic value, and therefore it is necessary to recover its valuable components in order to valorize it at its full potential (Barba et al., 2016). The capitalization of grape pomace can take many forms (Figure 4). Conventional methods applied for the valorization of grape pomace include distilling it, using it as fertilizer, or as animal feed, while modern applications include processing it in order to obtain biofuel, green materials, as well as bioactive compounds for the pharmaceutical, food and cosmetic industries (Chedea & Pop, 2019; Brezoiu et al., 2020).



**Figure 4:** Grape Pomace Applications

## **5. GRAPE POMACE IN HORTICULTURE AND SUSTAINABLE AGRICULTURE**

### **5.1. Grape Pomace As a Source of Biostimulants-Bioregenerators**

Currently, there is a growing interest for the usage of agricultural and horticultural residues in sustainable agriculture, as fertilizers, biopesticides and biostimulants. Among these easily available resources are fruit pomaces.

Biostimulants are products that contain chemical compounds and/or microorganisms that can be applied to soil or plant organs and that stimulate growth processes, nutrient uptake and transportation, tolerance to biotic and abiotic stress, flowering, fructification and general crop quality and yield. Biostimulant market is a growing one, with \$2 billion sales in Europe alone (Ertani et al., 2017).

One of the main advantages of using grape pomace is its quantity: over 6 million tons are produced yearly and most of it is lost as a waste that requires treatment (Kokkinomagoulos & Kandyliis, 2020).

One of the most important bioactive principles that make fruit residues valuable as biostimulants are phenolic compounds. These are potent antioxidants that, besides their importance in human and animal health, can also act as growth regulators, especially by stimulating the uptake of certain mineral macronutrients, such as potassium (Ertani et al., 2017). Seeds, which are an important part of pomace are rich in various phenolic antioxidants (catechins, cinnamic acid and derivatives, epicatechin, monomeric flavanols, gallic acid, gallocatechin, hydroxybenzoic acid, kaempferol, procyanidin dimers, trimers and polymers, quercetin), carotenoids, phytosterols, unsaturated fatty acids, vitamin E (Lucarini et al., 2018).

Apart from phenolic antioxidants (up to 1% or over), pomaces are rich in fibers (19-38%), various sugars (15-33%), lipids (0.4-1%), nitrogen (1-1.7%) and minerals (1.8-2.4%; Kokkinomagoulos & Kandyliis,

2020). Apart from directly supplying valuable compounds, pomace also indirectly stimulates the uptake of other nutrients (Artem et al., 2021). There are numerous methods of extraction of valuable chemicals from fruit tissue. Phenolic compounds can be extracted with ethanol or other solvents, usually at high temperatures and with long extraction times. Due to environmental and health concerns, there is a growing trend of searching alternative methods, including high pressure, ultrasound or supercritical fluid extraction. For protein-derived compounds, enzymatic hydrolysis (although traditionally used mostly for animal residues) is gaining attention as an effective method, with low environmental impact (Ertani et al., 2017).

Phenol-rich fruit residues are known for their biostimulative effects on crop plants. For instance, an experiment conducted on maize shown that fruit pomace hydrolysates resulted in significant increases in protein content and in various classes of phenolic compounds, although mixed results were obtained for sugars: sucrose, glucose and fructose, with major decreases in the former (Ertani et al., 2017).

More specifically, red grape skin residue extract (produced by cool extraction) applied on *Habanero pepper* plants (*Capsicum chinense*) led to an up to 80% increase in average leaf and fruit numbers and total fresh biomass. The treatment also increased the concentrations of fructose, glucose, ascorbic acid, dihydrocapsaicin, epicatechin, quercetin, chlorogenic acid and ferulic acid in leaves and that of capsaicin, glucose, ascorbate, fumarate, thymidine, chlorogenic acid, *p*-

coumaric acid, *p*-hydroxybenzoic acid, NADP<sup>+</sup>, and featured a higher antioxidant activity (Ertani et al., 2014).

Combined with other organic mixtures (algal residues, chopped vine shoots), grape pomace was found to help increase total biomass in several culture plants: grapevine, mustard, white clover – *Trifolium repens* (a forage plant) and ryegrass – *Lolium perenne* (a turf grass). In this experiment, both fermented and unfermented grape pomace and vine shoots were proven to be the most effective ingredients. Only part of this effect can be attributed to direct fertilization, humus enhancement or phosphorus and iron mobilization, bioactive growth-regulating compounds playing a similarly important role (Artem et al., 2021).

On citrus plants, on the other hand, application of grape pomace amendments was not found to bring significant benefits in terms of fruit size and total yield (Falivene, 2016).

Apart from direct or indirect plant growth enhancement, pomace is also useful against pests and pathogens. Alcoholic extracts of grape seeds added to soil release large quantities of free gallic acid and condensed tannins, which have nematicidal or nematostatic and antimicrobial properties (Lucarini et al., 2018).

White grape pomace extracts (using organic, environmentally-friendly solvents) were also successfully tested against *Phytophthora cinnamomi* (a parasitic oomycete causing chestnut ink disease, difficult to fight

against, since fungicide do not easily affect oomycetes), with inhibitory concentrations (IC<sub>50</sub>) as low as 3.17 % (Rama et al., 2020).

## **5.2. Grape Pomace As Ecological Fertilizer**

The aforementioned methods used for the recovery and recycling of grape pomace included using it as a fertilizer. Numerous times, research has shown that organic wastes can be turned into fertilizers, provided they are transformed in an appropriate way (Galanakis, 2017).

The organic cultivation system has become more and more prevalent nowadays, implying the use of fertilizers of natural origin instead of the synthetic ones (Arvanitoyannis & Varzakas, 2008). Graefe has proposed in 1982 a method of grape pomace recovery which involves using it as a high-grade organic fertilizer, also capitalizing on the heat and carbon dioxide produced.

The composting process has the aim of producing a homogenous, aerated, and reproductible fertilizing product, in well maintained conditions (Arvanitoyannis et al., 2006). Optimal thermal, pH, aeration and humidity conditions must be maintained in order to have a successful composting procedure (Dobrei et al., 2016).

Because of the potential presence of pathogens, long decomposing time, accompanied by the release of catabolites with an inhibitory action of the root growth and seed germination, it is not recommended to apply grape pomace as a fertilizer directly, without treating it properly (Owen, 2015). By decomposing, the unstable chemical compounds and the

activity of pathogenic microorganisms is reduced, thus making grape marc an adequate fertilizer (Dobrei et al., 2016).

Winemaking produces annually millions of tons of grape pomace as a byproduct, a revaluable resource with many potential uses, including a nutrient-rich organic soil amendment, but its application as untreated raw material can damage crops owing to the release of phytotoxic polyphenols, problem which can be minimized by vermicomposting process, as earthworms can partly digest polyphenols. The procedure is effective, simple, environmental-friendly and economical, and can easily be scaled up for industrial application yielding a variety of added-value products from the grape pomace (Domínguez et al., 2016).

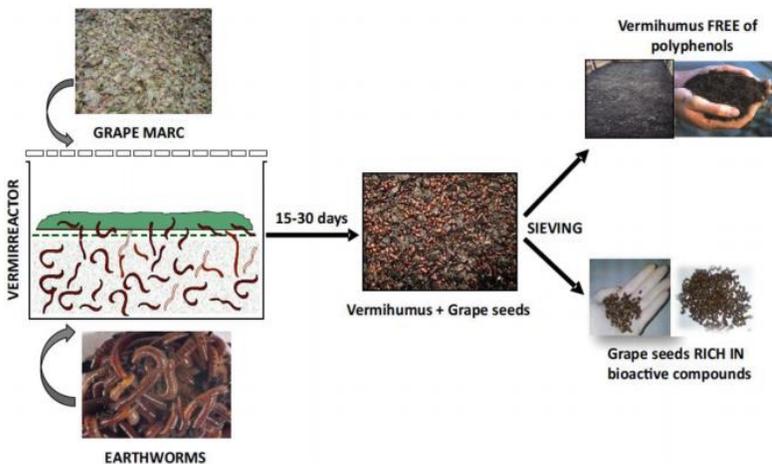
Composting and vermicomposting (Figure 5) are such methods of converting wastes resulted from various domains of agriculture, in order to obtain a satisfactory product for soil fertilization (Arvanitoyannis et al., 2006; Domínguez et al., 2015; Morata & Loira, 2016). Through these processes, the high polyphenolic content of the grape marc constituents is reduced, therefore minimizing their impact on the crops for which the fertilizer is applied (Figure 6) (Morata & Loira, 2016).

Grape pomace has the potential to become a valuable fertilizer, as it has good physicochemical properties and it can return nutrients back into the soil, Lasaridi et al. (2000) stating that this kind of composted material is of the best quality, due to its agronomic value.

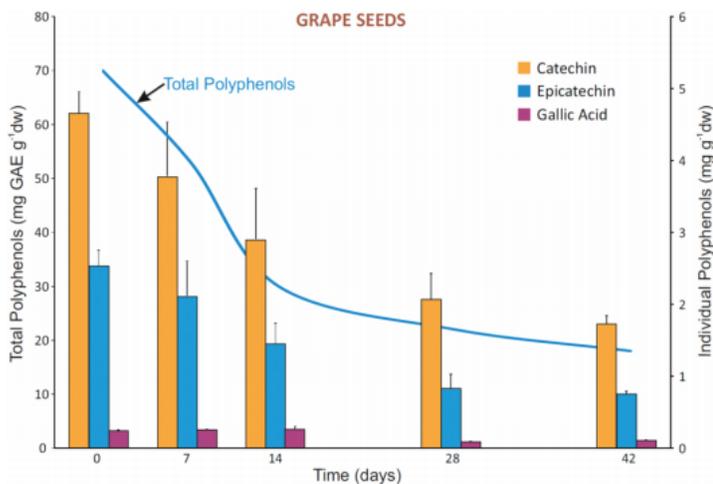
Chemical analyses have shown that grape pomace compost contains 2-3% w/w free potassium, the element present in the most notable amounts (Patti et al., 2009). However, the nitrogen (2.14-3.74%) and phosphorous (0.18-0.52%) contents are not that high (Table 1), and in addition, these nutrients are not found in a form that is available to plants.

Ferrer et al. (2001) recommend that the use of grape pomace compost should be supplemented with phosphorous, in order to satisfy crop needs for this nutrient.

Elements such as calcium, magnesium and sulphur are present in small amounts (Arvanitoyannis et al., 2006). Micronutrients like copper and boron are not present in excessive amounts and can satisfy the soil's needs for these elements.



**Figure 5:** The Scheme of Grape Pomace Biofertilizer Obtained by Vermicomposting Process to Obtain Simultaneously a High-Quality Biofertilizer Free of Polyphenols and Grape Seeds Rich in Bioactive Compounds (Domínguez et al., 2015; Morata & Loira, 2016)



**Figure 6:** The Effect of Compost Process on the Polyphenol Content of Grape Pomace (Morata & Loira, 2016)

**Table 1:** Chemical Composition of Compost Derived From Winery Waste (Arvanitoyannis & Varzakas, 2008)

ELEMENT	VALUES
<b>N</b>	2.14-3.74%
<b>P</b>	0.18-0.52%
<b>Ca</b>	3.17-14.3%
<b>Mg</b>	0.3-0.61%
<b>Fe</b>	0.5%
<b>Zn</b>	77-109 mg/kg
<b>Cu</b>	30-46 mg/kg
<b>Ni</b>	9.1-17.6 mg/kg
<b>Cr</b>	23.4-147 mg/kg
<b>Pb</b>	8-19 mg/kg
<b>Cd</b>	0.2-0.4 mg/kg

The organic carbon present in grape pomace compost was in the forms of lignin and carbohydrates, studies revealing the presence of the humification process (Patti et al., 2009). The use of this type of compost

implies the gradual release of nutrients, over a long period of time, an increase of the soil's organic matter content, also improving its microbial activity and physical characteristics, such as water-holding capacity and aeration (Ribereau-Gayon et al., 1982). This kind of fertilizer has become more sought after, as it can ameliorate soils characterized by erosion and low humus levels (Vilaseca et al., 1994), as well as having pollutant filtering capacities (Dobrei et al., 2016).

Grape pomace compost can be used on its own as a biofertilizer, as was mentioned before, or along with other components, which lead to a more potent effect. Compost made of freshly obtained grape pomace, combined with hen dropping can represent a higher quality fertilizer, compared with a synthetic product composed of  $P_2O_5$ ,  $K_2O$  and urea (Ferrer et al., 2001). The role of the addition of hen droppings in this case is to aid the aerobic and anaerobic bioconversion processes. This effect was produced due to the fact that the composted biofertilizer contains humic compounds, micronutrients and minerals (Arvanitoyannis & Varzakas, 2008).

In another study from 2004, Bertran et al. used winery sludge and grape stalks, in a ratio of 1:2 as a compost, obtaining a high value biofertilizer. This can be especially used for the vineyard soils, characterized by a low organic matter content, thus closing the cycle.

In a 2021 study on grapevine, Artem et al. have noticed an increase of grape quality, vegetative growth, as well as soil phosphorus and humus contents, after incorporating a mixture of grape pomace, grapevine

canes, *Rapana* sp. shells and green and brown algae into the soil (Negreanu-Pirjol et al., 2019).

The continuous use of grape pomace compost as a fertilizer can induce, however, undesirable effects, such as heavy metals bioaccumulation (Arvanitoyannis & Varzakas, 2008).

The abundance and availability of heavy metals and essential nutrients were evaluated in a comprehensively study by Pérez Cid et al., (2019), on different grape pomace residues from winemaking of different white and red grape varieties, before and after the distillation process and the data emphasize in this chapter, suggest the valuable application of grape pomace as eco-fertilizers for different agricultural soils, without limitations resulting from heavy metal contamination.

## **CONCLUSION**

Grape pomace obtained during the winemaking process, it is a mixture of skins, pulp, seeds, and stems, obtained after pressing of grapes in the winemaking process and represents about 20–30% wt. of processed grapes.

The valorization of grape pomace in circular bioeconomy is important considering the environmental issues or the important potential of its bioactive compounds, but in the last decade only 3% of grape pomace was turned into animal feed, compost, or other applications. In the same time, the grape pomace is an abundant and valuable source of polyphenolic compounds, which present an increased interest due to their health benefits, which involve antioxidant, anti-inflammatory,

antibacterial, anticarcinogenic, antidiabetic, cardioprotective effects and a valuable potential for food and cosmetics industries.

As a promising biostimulant, grape pomace boost crops yield and quality. Also, it improve the soil structure, increase the volume of useful land which give the crops better access to the soil's water and mineral resources, a better resistance and tolerance to water stress, increase the production and offer better crops physiological conditions.

As eco-fertilizer for plant nutrition, grape pomace can help make soils more fertile by restoring a level of organic matter needed for healthy plant growth through the complexed micronutrients content and also, could reduce abiotic stress caused by drought or high soil salinity.

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## CHAPTER VI

### MARINE ALGAE AN IMPORTANT RESOURCE FOR BIOECONOMY AND BIOMEDICAL APPLICATIONS.

#### A REVIEW

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## **INTRODUCTION**

Macroalgae are commonly found in marine habitats including seas and oceans and known as seaweeds, while a few species could grow and proliferate in other freshwater ecosystems (Ibraheem et al., 2014). Macroalgae are classified into three classes: green algae (Chlorophyta), brown algae (Phaeophyta) and red algae (Rhodophyta). The characteristic green colour of green algae is due to the presence of chlorophyll a and b as in higher plants. The brown pigmentation of phaeophytes is attributed to the dominance of xanthophylls and fucoxanthin pigments which mask other pigments. Phycoerythrin constitutes the major pigment in rhodophytes (red algae) and is mainly responsible for giving the red colour (Abad et al., 2011).

Marine algae are plant-like organisms with simple internal structures that generally live in coastal areas. Marine algae commonly occupy intertidal and sublittoral-to-littoral zones on rocks and other hard substrata (Lewin & Andersen, 2019). They are considered to be an excellent natural biosource in different aspects of horticultural fields. They have great proficiency in improving soil physical and chemical properties (Newton, 1951). Unfortunately, most available literatures on marine algae and their derivatives mainly focused on their pharmaceutical applications but their potential utilization in sustainable horticulture and agriculture development is still often regarded as a secondary goal. However, a relatively considerable dataset on marine algae showed that they could play a major role in plant protection and

improvement. This review summarizes different aspects of algal applications in horticulture, industry and biomedical domains.

Marine macroalgae extracts also have been used in agricultural trends as soil conditioners to enhance crop productivity (Abdel-Raouf et al., 2012). Macroalgal-extracted polysaccharides also have been confirmed to be used as perfect metal ion chelators. Furthermore, it has been reported that these polysaccharides are rich in functional groups having the ability to bind to some micro elements with important plant nutritional value (Kaplan et al., 1987). They are also well known as plant stimulators-bioregenerators.

They have been applied as foliar spray, enhanced plant growth at freezing, drought and in salt habitats, showed a noticeable strong resistance to fungi, bacteria and virus and also improved the yield and productivity of several crops (Eris et al., 1995; Norrie & Keathley, 2006; Gajc-Wolska et al., 2013; Sharma et al., 2014).

Marine algae are made up of sulfated polysaccharides but also proteins, lipids, minerals, vitamins, pigments and other secondary metabolites. Therefore, algae have proved to be a good solution for plant growth. Recent studies show that they have very good results in terms of plant nutrition and, also, that they can be used to develop algae-based biostimulants and elicitors thanks to their unique properties:

- Lipids and sugars will provide plants with energy.
- High protein content will bring a wide spectrum of essential aminoacids.

- Minerals, which are basic for the structure of the plant.
- Vitamins for a well-functioning.

Sustainable horticulture is advantageous over conventional agriculture for its capacity to accomplish food demand by utilizing environmental resources without negatively affecting it. The beneficiary role of algae by way of supporting the nitrogen economy of paddy fields and enhancing rice productivity is well known. The simple presence of algae in soil results in formation of soil aggregates, which reduces soil loss during rainy season and regulates aeration, soil temperature, hence, improving physical and chemical properties of soil vis-a-vis physical environment of the crop. Marine algae are helpful in restoring soil nutrients by secreting exopolysaccharides and bioactive substances. They have the potential to mobilize insoluble forms of inorganic phosphates. Algalization has been employed for reducing the amount of exchangeable sodium, which results in altered soil pH and leads to reclamation of sodic soils. Some red algae used as biofertilizers have been found to augment growth nutritional value and yield of crop plants. This review also provides an overview of the role of algae in horticulture and agriculture, as biofertilizers. (Chatterjee et al, 2017).

## **1. THE ROMANIAN COASTAL ZONE OF THE BLACK SEA**

For ecological studies, the coastal zone represents a special interest because it is the area with the highest values of biological production, as the variation of parameters in the abiotic environment is both

dynamic and unpredictable (storms, wind intensity and direction, variations of temperature and of maximum isolation period etc).

The coastal zone represents the geographical space situated at the contact between sea and land and includes the surface and underground coastal waters, as well as the adjacent lands (strongly inter-conditioned and nearby the shoreline), islands and salt lakes, wetlands in contact with the sea, the beach and the seawall.

The Romanian littoral of the Black Sea has a length of 245 km (6 % of the total length of the Black Sea shore), between the flowing mouth of the Chilia Branch, to the border with Ukraine in the north and with Bulgaria in the south.

The Romanian coastal area is divided into two geographical and geomorphic units:

- The northern unit (N) occupies 2/3 of the total littoral length and stretches between Musura Bay, at the flowing mouth of the Chilia Branch and Cape Singol, including the shore of the Danube Delta Biosphere Reservation;
- The southern unit (S) occupies 1/3 of the total length of the Romanian littoral and stretches between Cape Singol and Vama Veche.

The frequency of episodes with macroalgal deposits is higher in the southern sector (and over longer periods of time), a fact explained by the very presence of submerse limestone platforms (Figure 1).



**Figure 1:** Macroalgal Deposit along Romanian Black Sea Coast (*Ulva* and *Enteromorpha* species) (Negreanu-Pirjol et al., 2016)

The macrophytobenthos is made up of macroscopic algae (green, brown and red) and phanerogams. It is spread over the north-western continental platform of the Black Sea basin (in littoral zones of low depth) and it can sometimes form important deposits at the shore, especially in the pre-vernal or vernal season or after storms (Negreanu-Pirjol et al., 2016).

In the recent years, as a result of the recovery trend of the marine macroflora and due to the specific meteorological and lithological conditions of the littoral zone, the forming of algae deposits on the beach has been observed. The studies concerning the possibility of utilization of the marine algae deposits along the Romanian seaside have been carried out. Two directions were taken into account:

- Utilization of the marine algae in agriculture - soil fertilizer;
- Marine biotechnology - researches for extraction and purification of some biological active substances from marine algae.

Due to their nutritional characteristics, the macroalgae from the Black Sea coast represent a natural resource important for food to humans, as fodder to animals, in agriculture as fertilizer, as ingredients in food and cosmetics industries, as a source for substances with pharmacodynamics actions (Marin et al., 2016).

The '80s represent a period of increased eutrophication and pollution, which generated important modifications in the qualitative structure of macrophytobenthos.

The effects of these modifications are still felt today (Bologa, 2001; Sava et al., 2007), 86 macroalgal species were cited in the 1970s-1980s (Bavaru, 1977), 69 species were cited in the 1980s-1990s and only 55 species after 1990 (Vasiliu, 1984; Vasiliu, 1996). This decrease in the number of species (apart from the pollution phenomenon) was attributed to climate changes – frost at the Romanian littoral (for unprecedented long periods of time) on the one hand and to the deposits of clay sediments on limestone platforms (fraction mobilized and brought to the sea mass as a result of hydrotechnical constructions in the Harbors of Cape Midia and Agigea South) on the other hand.

## **1.1. The Taxonomic Structure of Macroalgal Communities at the Romanian Littoral**

### **1.1.1. Green Algae – Chlorophyta**

Distributed over the entire length of the Romanian sector, from south of the Danube flowing mouths to the southern extremity of the littoral (Vama Veche, Constanta County), green algae species are more

developed in the Cape Singol-Constanta to Vama Veche sector, Romania. The mass development of the macroalgal carpet in this sector is explained by the very presence of limestone platforms which cover almost entirely the shallow waters and which represent the ideal substrate for their attachment (Negreanu-Pirjol et al., 2016).

The group includes representatives of Chlorophyceae, three orders: Ulvales, Cladophorales and Bryopsidales; the vegetative apparatus is diverse: lamellate, filamentous, tubular or cladomial.

Green species *Ulva lactuca* syn. *Ulva rigida*, C. Agardh, 1823 (Figure 2) and *Cladophora vagabunda* (Linnaeus) Hoek, 1963 (Figure 3), are considered opportunistic species, with a high reproductive capacity and a frequent distribution at the Romanian shore, with an abundant development between 0-3 meters depth (Rosioru et al., 2018).

*Ulva rigida* dominates the hard substrate at the Romanian Black Sea coast, developing a considerable fresh biomass especially during the warm season.

*Cladophora* is a genus of reticulated filamentous Ulvophyceae, species with a high ecological amplitude and can withstand into the most diverse ecological conditions. These aspects demonstrate the opportunistic character of the species and its invasive tendency (Marin & Timofte, 2011).



**Figure 2:** *Ulva lactuca* syn. *Ulva rigida*  
(Marin & Timofte, 2011)

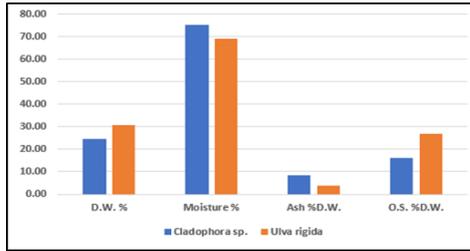
**Figure 3:** *Cladophora vagabunda*  
(Marin & Timofte, 2011)

The biochemical composition of the sea organisms are not constant. They are related to the life cycle and external factors, like temperature, salinity, and nutrition. The geographical origin of the organisms is an additional factor that affects the composition of sea organisms (Rosioru et al. 2018).

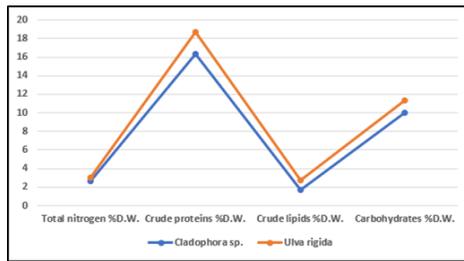
The physical-chemical composition of green and red marine algae was assayed by Negreanu-Pirjol B. et al. (2011 a).

The biochemical composition (DW-dry wet, moisture, ash, organic substance, total nitrogen, crude proteins, crude lipids, carbohydrates, chlorophyll a, chlorophyll b, carotenoids) and dietary fiber (CF-crude fiber, ADF- acid detergent fiber, NDF- neutral detergent fiber, NDS-neutral detergent solubles, ADL- acid detergent lignin) were assayed in *Ulva rigida* and *Cladophora* sp., in 2020.

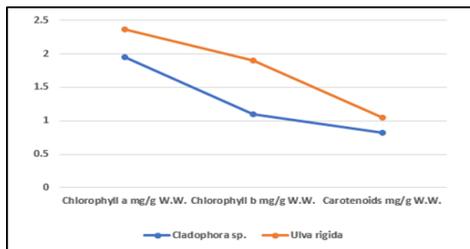
The results are summarized in Figure 4, Figure 5, Figure 6 and Figure 7. (Rosioru D.M., unpublished data).



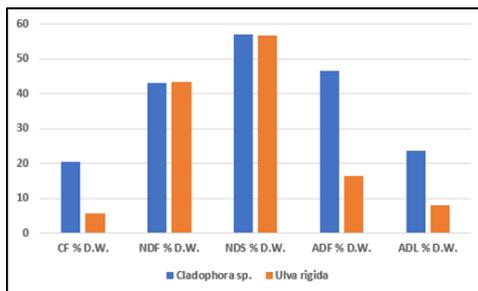
**Figure 4:** Global Biochemical Content of *Ulva rigida* and *Cladophora* sp.



**Figure 5:** Bioactive Biochemical Compounds of *Ulva rigida* and *Cladophora* sp.



**Figure 6:** Chlorophyll (a, b) and carotenoids content of *Ulva rigida* and *Cladophora* sp.



**Figure 7:** Fiber Content: CF, ADF, NDF, NDS, ADL of *Ulva rigida* and *Cladophora* sp.

The heavy metal concentrations were analyzed in *Ulva rigida* and *Cladophora* sp. from the Black Sea (Cadaru et al., 2019; Negreanu-Pirjol et al., 2011b) and Table 1.

**Table 1:** Heavy Metals Concentrations in the Two Species of Green Macroalgae from Romanian Black Sea Coast in 2018 in Comparison with Previous Studies (2016) and Data Reported in Other Regions (Rosioru et al., 2018)

Species	Location	Cu ( $\mu\text{g/g}$ d.w.)	Cd ( $\mu\text{g/g}$ d.w.)	Pb ( $\mu\text{g/g}$ d.w.)	Ni ( $\mu\text{g/g}$ d.w.)	Cr ( $\mu\text{g/g}$ d.w.)	References
<i>Ulva rigida</i>	Romanian Black Sea, Vama Veche	7.92	0.38	0.013	4.05	3.373	Rosioru et al., 2018
<i>Cladophora</i> sp.	Romanian Black Sea, 2 Mai	11.41	0.53	0.63	13.80	22.06	Rosioru et al., 2018
<i>Ulva rigida</i>	Romanian Black Sea, Agigea	17.03	0.32	1.63	24.37	1.06	Oros A, 2016, unpublished data
<i>Cladophora</i> sp.	Romanian Black Sea, Eforie Nord	33.57	3.74	1.32	5.52	2.01	Oros A, 2016, unpublished data
<i>Green macroalgae (Chlorophyta)</i>	Turkish Black Sea	2.09 – 24.10	0.02 – 3.30	0.01 – 9.3	0.1 – 31.50		Bat, 2014

### 1.1.2. Brown Algae – Pheophyta

Brown algae prefer cold marine waters, developing exuberantly at greater depths than green algae. Representatives from three classes are found at the Romanian littoral: Isogeneratae (with two species of *Ectocarpus* and one species of *Scytosiphon*), Heterogeneratae (Punctaria) and Cyclosporeae (with the most representative species at the Romanian littoral from this group, *Cystoseira barbata*, Good et Wood, Ag., 1821 (Figure 8).



**Figure 8:** *Cystoseira barbata*, Good et Wood, Ag., 1821  
(Original by Negreanu-Pirjol)

The representatives of the first two classes register reduced biomasses compared to the representatives of green algae, especially in spring and autumn. In the Black Sea, *Cystoseira barbata* forms a perennial association fixed to the hard substrate, especially in the southern sector of the Romanian littoral. If in the past it was one of the most important associations, covering the infralittoral zone between Agigea-Vama Veche, Constanta County, Romania, it is currently much reduced (due to the frost periods in 1975 but also to pollution, increased water turbidity and substrate clogging). The algae biomass present in deposits at the seashore is relatively reduced, but its importance is given by the fact that its relatively rigid thallus constitutes a substrate for a rich subaquatic fauna and for epiphytic algae (Figure 9).



**Figure 9:** Algal Carpet Made up of Species of the Genera *Ulva*, *Enteromorpha* and *Ceramium* (Negreanu-Pirjol et al., 2016)

### 1.1.3. Red Algae - Rhodophyta

The representatives of this group make up the phytobenthos in the deep zone of the infralittoral. The algal deposits register an increased biomass compared to brown algae, but reduced in comparison to green algae. Two classes have representatives in the Black Sea:

- Bangiophyceae (with *Porphyra leucosticta* Thur and *Bangia fuscopurpurea* Lyngb. Both are cold water species and grow at the end of winter till March without developing significant biomasses, compared to green algae);
- Florideophyceae – with the perennial species, *Hildenbrandtia rubra* Menegh. (distributed in the shallow zone, attached to rocks or to mollusk shells). The species *Corallina officinalis* L. (pharmaceutical importance – vermifuge) develops in the shallow infralittoral - permanently covered by water - and shelters a characteristic fauna. It rarely occurs in shore algal deposits. Four

species of the genus *Phyllophora* form “Zernov’s field” in the north-western basin of the Black Sea.

This field suffered a significant reduction but clusters that form associations specific to the invertebrate fauna can still be encountered. The biomass is reduced in the shore deposits. There are four representatives of Ceramiaceae: *Callithamnion corymbosum* Lyngb. and three species of the genus *Ceramium* (these develop significant biomasses in the shallow zone – depths between 1.5 to 4-5 m), in the prevernal and vernal season. One species from Rhodomelaceae – *Polysiphonia denudata* Grev. encountered in the warm season but without significant biomasses.

## **2. BIOLOGICAL ACTIVITIES OF MARINE ALGAE**

Marine algae are ubiquitous micro- and macroscopic species with various ecological roles e.g. bioindicators, bioremediators, biofertilizers and therefore bioreservoirs of active compounds with biomedical applications and cosmeceutical potential (Sirbu et al., 2010; Negreanu-Pirjol et al., 2018a). Adaptation to environmental factors such as temperature, salinity, pH, sunlight exposure, CO<sub>2</sub> supply modulate the chemical composition of marine algae (Chew et al., 2020; Wang et al., 2020).

## **2.1. Active Compounds Derived From Algae**

### **2.1.1. Polysaccharides**

Polysaccharides are the main constituent of the algae (Ekelhof & Melkonian, 2017; Gainard et al., 2018). The complex macromolecules are built in general from repetitive monosaccharide units linked with glycosidic bonds, while some exhibit a linear backbone consisting of repeating disaccharide units (Perez et al., 2016; Xu et al., 2017). Algal polysaccharides are involved in the structural support as well as storage being mainly found in the cell walls. Alginate, fucoidan and laminarin are the predominantly encountered in brown algae. Green algae are abundant in ulvan and cellulose, with additional constituents such as amylose, amylopectin, inulin, mannan, pectin, and xylan. Ulvan has a molecular weight in the range of 89 to 8200 kDa and is a water-soluble polysaccharide (Mo'o et al., 2020). Alginates are composed of  $\alpha$ -1-guluronic acid and  $\beta$ -D-mannuronic acid with a molecular weight ranging from 500 to 1000 kDa (Moenne & Gonzalez, 2021). Carrageenan and agar are found in red algae. Carrageenans are linear polysaccharides chains with attached sulphate half-esters. Depending on the degree of molecular sulphation, carrageenans are classified into three forms: kappa, lambda and iota (Vera et al., 2011). Agars comprise a mixture between agarose and smaller molecules agaropectin (Kumar et al., 2013).

### **2.1.2. Fatty Acids**

Algae are critical source of very long-chain polyunsaturated fatty acids (PUFAs), which are essential for human and also animal health. A high content of PUFAs is usually found in macroalgae from cold waters. Alpha-linolenic and linoleic acids are PUFAs basic precursors and were recognized almost 100 years ago as critical for animal diet (Burr & Burr, 1930). The main constituents of the algal lipidic fractions are omega-3 fatty acids: eicosapentaenoic acid and docosahexaenoic acid and omega-6 fatty acid:  $\gamma$ -linolenic acid and arachidonic acid (Zhang et al., 2019). PUFAs exhibit multiple health benefits such as regulation of blood pressure and blood clotting, decreasing the risk of chronic diseases (arthritis, diabetes, obesity) (Layé et al., 2018).

### **2.1.3. Phenols**

Phenols are defined by hydroxyl groups directly linked to aromatic hydrocarbon rings. Phenolic compounds have different chemical structures, one aromatic ring – phenol, or multiple phenol units – polyphenols. Despite, their large distribution in the Plant kingdom, phenols derived from marine algae are different. The predominant polyphenols found in marine algae are phloroglucinols and phlorotannins. Phlorotannins are divided in different subclasses: eckols, fuhalols, fucophloorethols, phlorethols, fucols and ishofuhalols. Green and red algae are the most abundant in phenolic compounds (phenolic acids, bromophenols and flavonoids), while marine brown algae contain exclusively phlorotannins.

Marine algae with a high content of phenols are beneficial alternatives with applications in nutraceutical sector. Phenols and polyphenols are promising compounds with antitumoral, antioxidant, antimicrobial and inflammatory activities (Ghosh et al., 2019).

#### **2.1.4. Proteins and Amino Acids**

Traditional algae rich diet specific to Asian populations has consistently linked to a lower incidence of chronic maladies such as cancer or cardiovascular diseases. They are considered a viable protein source, with essential amino acid composition requirements and superior to that of other plant sources (wheat or rice) (Barbier et al., 2019). Algal protein content is predominantly rich in aspartic and glutamic acid. Glutamate is the major component of the savory, the fifth basic taste called umami. *Spirulina* (*S. platensis*) and different species of the unicellular green alga *Chlorella* are the main species cultivated worldwide for production due to their content of up to 70% protein as dry weight.

Green macroalgae and particularly red macroalgae are more abundant in proteins when compared to brown ones (Wells et al., 2017). A series of bioactive amino acids and peptides, such as taurine, carnosine, glutathione and mycosporine-like have been showed to exhibit antioxidant properties and antiapoptotic effects in *in vivo* studies (Aydm et al., 2016).

**Table 2:** The Active Compounds Derived From Marine Algae

Active compound	Biological activity	Mechanism of action	Algae species	Reference
Astaxanthin	Antiproliferation	ROS scavenging potential	<i>Haemotococcus pluvialis</i>	Rao et al., 2013
	Anti-aging	Inhibition of MMP expression		Shin et al., 2017
Fucoidan	Anti-inflammation	Inhibition of LPS induced NO production, COX-2 and PGE2 levels	<i>Chnoospora minima</i>	Fernando et al., 2017
	Anti-proliferation	Inhibition of cell proliferation	<i>Fucus evanescens</i>	Anastyuk et al., 2012
		Decrease of melanoma growth		<i>Fucus vesiculosus</i>
	Anti-melanogenic	Inhibition of tyrosinase and melanin		
Fucoxanthin	Antioxidant	DPPH inhibition	<i>Cladosiphon okamurans</i>	Mise et al., 2011
			<i>Hizikia fusiformis</i>	Yan et al., 1999
		Prevention of oxidation	<i>Fucus vesiculosus</i>	Zaragoza et al., 2008
Dieckol	Anti-inflammation	Suppression of iNOS and COX-2		Jung et al., 2009
Phlorotannin	Anti-melanogenic	Inhibition of melanin production	<i>Ecklonia cava</i>	Yoon et al., 2009
		Downregulation of tyrosinase and melanin synthesis	<i>Ishige foliacea</i>	Kim et al., 2013
	Antioxidant capacity	ROS scavenging potential	<i>Ecklonia cava</i>	Kang et al., 2005
	Anti-inflammation	Inhibition of hyaluronidase	<i>Ecklonia kurome</i>	Shibata et al., 2002
			<i>Eisenia bicyclis</i>	
	Anti-aging	Inhibition of NNP expression	<i>Eisenia arborea</i>	Sugiura et al., 2006
<i>Ecklonia stolonifera</i>			Joe et al., 2006	
Polyphenol	Anti-microbial	Bacterial growth inhibition	<i>Padina pavonica</i>	Saidani et al., 2012
	Antioxidant capacity	Antioxidant capacity	<i>Laminaria ochroleuca</i>	Del Olmo et al., 2019
		DPPH inhibition	<i>Cystoseira foeniculacea</i>	Messina et al., 2019
Sulfated polysaccharide	Anti-inflammation	Inhibition of COX-2 and iNOS	<i>Padina tetrastrumatic</i>	Mohsin & Kurup, 2011
		Inhibition of LPS inflammatory reaction	<i>Sargassum hemiphyllum</i>	Hwang et al., 2011

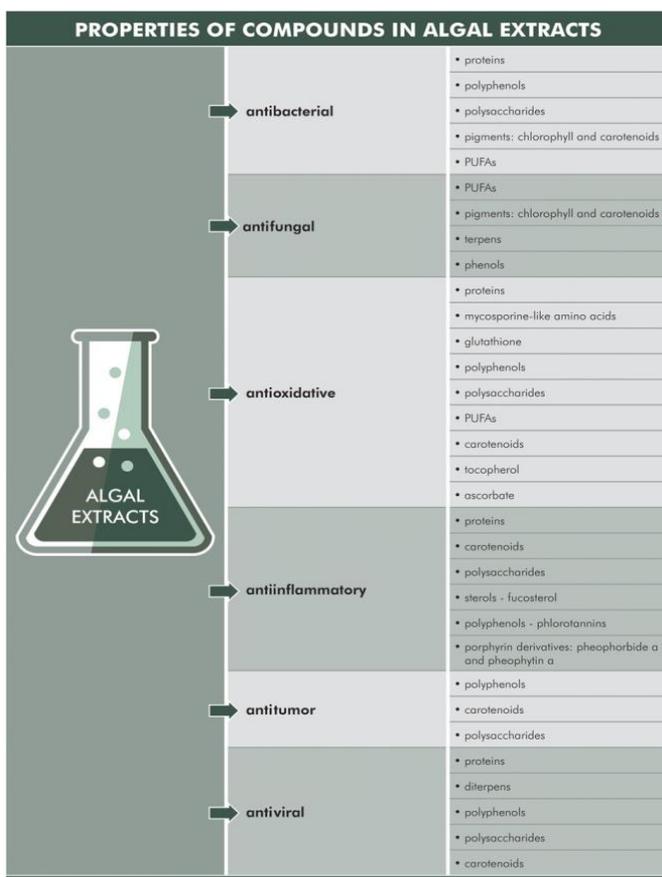
	Anti-proliferation	Activation of caspase 3	<i>Sargassum henslowianum</i>	Ale et al., 2011
Phenol	Antioxidant capacity	DPPH inhibition	<i>Ulva rigida</i>	Fernandes et al., 2019
			<i>Ulva prolifera</i>	Farasat et al., 2013
		DPPH, beta-carotene bleaching and ABTS inhibitions	<i>Pyropia columbia</i>	Cian et al., 2019
	Anti-melanogenic activity	Inhibition of tyrosinase activity	<i>Schizymenia dubyi</i>	Tan et al., 2019
Fatty acid	Anti-microbial	Bacterial growth inhibition	<i>Planktochlorella nurekis</i>	Shannon, & Abu-Ghannam, 2016
Polysaccharide	Anti-aging	Collagen synthesis	<i>Pyropia yezoensis</i>	Kim et al., 2017
	Antioxidant capacity	DPPH inhibition	<i>Laurencia obtusa</i>	Lajili et al., 2019
			<i>Chondrus canaliculatus</i>	Jaballi et al., 2019

Lectins are a valuable class of glycoproteins that due to their ability to bind to specific glycan structures (Coelho et al., 2017) manifest various beneficial effects including anti-inflammatory, mitogenic, antinociceptive and anti-viral (Silva et al., 2010). An important aspect is the seasonal variation, respectively the summer limitation of nutrients that affects the relative proportions of different amino acids (Wells et al., 2017). Yet, marine algae are considered a precious source of bioactive peptides with considerable horticultural and biomedical potential.

Different active compounds identified as therapeutical principles in marine algae, are emphasize in Table 2.

## 2.2. Biomedical Properties

Algae, the basis of marine food chain, produce a wide variety of biochemically active compounds with antioxidant, anti-inflammatory, antiviral, antibacterial, antifungal, hypercholesterolemia and hypolipidemic and antineoplastic effects (Sirbu et al., 2019; Michalak & Chojnacka, 2015) (Figure 10).



**Figure 10:** Biomedical Properties of Marine Algae Extracts (Michalak & Chojnacka, 2015)

### **2.2.1. Antioxidant Properties**

Oxidative stress is described as an imbalance between the free radicals generated by the prooxidant reactions and the antioxidant mechanisms. The increased production of free radicals is closely linked to diverse pathologies that comprise AIDS, diabetes, atherosclerosis, neurodegenerative diseases, carcinogenesis as well as natural aging and apoptosis processes (Masutani, 2000).

Significant antioxidant properties have been indicated for seaweeds in clinical and experimental studies (Craig, 1997; Negreanu-Pirjol T. et al., 2012; Negreanu-Pirjol B.S. et al., 2015; Attiq et al., 2018). Hence the increased interest in the study of seaweeds as sources of bioactive compounds given the background of traditional diet in diverse populations, yet not used as phytodrugs (Jiao et al., 2011; Athiperumalsami et al., 2011; Sirbu et al., 2019).

Secondary metabolites with antioxidant properties can be obtained by various extraction methods. For instance, Senevirathne et al. (2006) valuated seven different fractions of *Ecklonia cava* and observed that the polar fraction was the richest in phenolic content and expressed the strongest reducing capacity.

It is widely recognized that polyphenols are the main source of antioxidants in seaweed species (Salehi et al., 2019). There are various studies that report the polyphenolic content derived from seaweeds as for example phenolic acids, phlorotannins, flavonoids or bromophenol (Gutierrez et al., 2015). In addition, various bioactive compounds

contribute to the algae antioxidant properties such as: apolar compounds derived from chlorophyll, terpenoids or carotenoids, lipo- and hydro-soluble vitamin E and C, aminoacids (mycosporine-like and scytonemic) able to absorb appreciable UV radiation thus preventing peroxidation (Rastogi et al., 2017). Seaweeds are exposed to a high incidence of UV radiation, suggesting the development of an efficient system of antioxidant defence (Ramos et al., 2018).

### **2.2.2. Anti-Inflammatory Properties**

Inflammation is a complex physiological process that leads to immune system activation and is essential in host defense against pathogens. Still, chronic inflammation at subclinical level when prolonged and unregulated can damage host tissue with repercussions on chronic conditions already installed such as cardiovascular disease (Ridker et al., 2017), inflammatory bowel disease (Singh et al., 2016), obesity (Smith et al., 2018) and Alzheimer's disease (Heneka et al., 2018). The use of synthetic pharmacological compounds has shown promising results in limiting inflammation present in metabolic pathologies. Yet, long term use of anti-inflammatory drugs is correlated with adverse gastrointestinal effects (Wong, 2019). Dietary natural bioactives are desirable alternatives as anti-inflammatory agents with limited associated secondary symptoms. For instance, algae have an abundant potential with a 2-5% lipidic content on a dry weight basis, where up to 70% of the total fatty acids are PUFA. There are studies which report the anti-inflammatory activity of long chain n-3 PUFA (n-3 LC-PUFA) both *in vitro* and *in vivo* (Cândido et al., 2018). The mechanism of

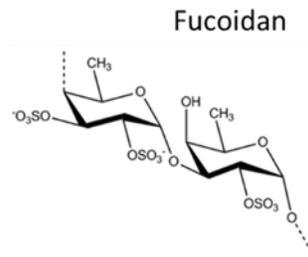
action is the empowering of the anti-inflammatory mediators, eicosanoids, which inhibit pro-inflammatory cytokine (interleukin-6 and interleukin 1 $\beta$ ) production at gene and protein level. There are several studies to support the evidence of algal extracts as functional food to modulate inflammation. Banskota et al. found that lipid extracts of *Tetraselmis chuii*, *Chlorella sorokiniana* and *Chondrus crispus* reduced inflammation in the mouse macrophage cell line RAW 264.7 (Banskota et al., 2014). Extract of microalgae *Nannochloropsis oculata* was reported to induce an anti-inflammatory effect on RAW 264.7 cells. The anti-inflammatory activity was obtained by the inhibition of iNOS and COX-2 proteins (Sanjeeva et al., 2016). It was shown that microalgae-derived bioactive compounds are suitable anti-inflammatory agents in skin disorders (psoriasis, vitiligo, atopic dermatitis) due to their tremendous structural diversity and biological availability. In addition, a significant advantage is the manipulation of the growth conditions (addition or removal of specific nutrients) for algae in order to enhance the production of target biocompounds (Fu et al., 2017). Green marine algae *Caulerpa mexicana* exhibit in vitro and in vivo anti-inflammatory effects such as the limitation of cell migration and edema formation. Bitencourt et al. reported the anti-inflammatory activity (reduced levels of cytokine from Th1 and Th17) of methanolic extract of this seaweed in an in vivo model of colitis (Bitencourt et al., 2015).

### 2.2.3. Anti-Tumoral Properties

Today, there are eight antitumoral drugs approved by the European Evaluation Medicines Agency (EMA), the US Food and Drug Administration (FDA), or the Australian Therapeutic Goods Administration (ATGA) derived from marine environment, and additionally in phase I, II or III clinical trials (Pereira, 2019).

Among the algal bioactive compounds, sulfated polysaccharides, carotenoids and phlorotannins showed high efficiency in antitumoral strategies management (Kim et al., 2011; Wang et al., 2020). The majority of the in vivo studies concerning the anticancer potential are focused on sulfated polysaccharides. Fucoidan (Figure 11), one of the most promising compounds from this class, is found in the cell wall of various brown macroalgae (Phaeophyceae). It was shown, both in in vitro and in vivo studies, to exhibit antiproliferative effect against a broad spectrum of cancers, including lung (Hsu et al., 2018), breast (Hsu et al., 2013) and head and neck (Mizrachi et al., 2017) cancers. For human use, it is administrated as adjuvant in standard chemotherapy (Ikeguchi et al., 2011). Nevertheless, further research is needed due to the highly complex chemical structure dependent on the biological origin and with significant echoes in its potency. H3-a1 is another sulphated polysaccharide derived from the brown seaweed *Hydroclathrus clathratus* that proved to reduce in vivo tumor growth of murine ascitic sarcoma and prolonged mice life span (Wang et al., 2010). Similarly, the alkali-extracted polysaccharide DAEB from the green algae *Enteromorpha intestinalis* induced inhibitory effect in the

same cancer model. The antiproliferative effects are triggered by the immune-stimulatory properties of the bioactive compounds through TNF- $\alpha$  induction (Jiao et al., 2009). In addition, polysaccharide with anti-metastatic potential was isolated from a brown alga, MSP that suppressed the metastasis development in vivo in a model of Lewis lung carcinoma (Tang et al., 2006). Then as well, calcium spirulan derived from *Spirulina platensis* limited the growth of the spontaneous melanoma lung metastasis model B16-BL6 (Mishima et al., 1998).



**Figure 11:** Chemical Structures of Algal Polysaccharide with Antiproliferative Potential

#### 2.2.4. Antimicrobial Properties

The emerging critical resistance of pathogens impedes effective prevention and treatment of various infections caused by bacteria, parasites, viruses and fungi. Despite being a natural phenomenon, today we are facing the antimicrobial resistance crisis, with a alarming limitation of effectiveness in antibiotic therapy and important consequences in immunosuppressive treatments or successful surgical procedures. The misuse and overuse of antimicrobials in clinics is one of the main triggers of nosocomial settings. In addition, the antibiotic

use in livestock farming (Zhao et al., 2020), aquaculture (Lulijwa et al., 2020), and the uncontrolled discard of antibiotics in the environment (Hassoun-Kheir et al., 2020) supports the horizontal transmission of antibiotic-resistance process.

Therefore, there is an urgent need for new natural compounds free of toxicity to humans and the environment with antimicrobial properties (Negreanu-Pirjol et al., 2018b). Marine algae are referred as attractive source of novel antibiotics. The reported antimicrobial activity of their polyphenols has great potential for designing innovative potent pharmaceuticals (Sirbu et al., 2010). The interest for antimicrobial agents derived from microalgae goes back to 1940s with the study of chlorellin obtained from *Chlorella*. Pratt et al. (1944) reported the obtention of a mixture of fatty acids (chlorellin) with antibacterial effects on both Gram-positive and Gram-negative bacteria (Pratt et al., 1944). Different microalgal antimicrobial compounds emerged between 1950s and 1980s such as two chlorophyll a derivatives (Senhorinho et al., 2015). The main molecules with antimicrobial potential refer to short chain fatty acids, monosaturated and unsaturated long chain fatty acids, as well as a variety of chemical compounds such as phenols, terpenes, pigments and indoles, acerogenins, alkaloids, macrolides, peptides and volatile halogenated hydrocarbons (Falaise et al., 2016; Shannon & Abu-Ghannam, 2016; Bashir et al., 2018).

Polyphenols and more specifically phlorotannins are a consistent group of algal bioactive compounds with biomedical potential (Poole et al., 2019). Phlorotannins are a heterogenous group of molecules with

various structure and degree of polymerization, on which limited information is available (Imbs & Zvyagintseva, 2018). Similar to the tannins derived from terrestrial plants, these phenols have a polymer structure, are highly soluble in water, bound to proteins, polysaccharides and other biopolymers and chelate divalent metals. The structure has the phloroglucinol as monomeric unit (1,3,5-trihydroxybenzene) and there are more than 700 natural variations. Genus *Fucus* has the largest amounts of phlorotannins, up to 3-12% of dry weight (Catarino et al., 2020). With a wide molecular weight range (10-100kDa), and a large difference of isomerization levels, it is difficult to obtain complete characterization. While almost 90% of the total phlorotannins is found in a free state in membrane-bound vesicles (physodes), the rest of the polyphenols are found in the cell wall bound in a complex with alginic acid and act as a structural component with role in regulating osmotic pressure (Koivikko, 2008). Some phlorotannins can be found in a sulfated or halogenated state, their biosynthesis being performed in the Golgi apparatus in the perinuclear region of the cell, with extraction possible only via cell destruction (Moon et al., 2008). Concerning the structure and polymeric characteristics, phlorotannins are an extensive group of molecules that differentiate due to the bonds among the phloroglucinol units and the number of hydroxyl groups. The pharmacological potential of polyphenols is linked to the specific structure and in particular the degree of polymerization, as for the antioxidant capacity an important characteristic seems to be the molecular weight. Phloroglucinol, eckol

and dieckol have been reported to be the typical phlorotannins with antimicrobial properties (Moon et al., 2008; Suleria et al., 2015).

Antimicrobial properties screening of different algae revealed efficient compounds against both bacterial and fungal strains. Boonchum et al. (2011) reported inhibitory effect on different bacteria organisms *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Propionibacterium acnes* and *Proteus mirabilis* and fungal strain *Candida albicans*. The evaluation of the antimicrobial properties of extracts from *Dunaliella tertiolecta* and *Pseudokirchneriella subcapitata* against the etiological agents of otitis revealed antibacterial effect especially against *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Pane et al., 2015). The antibacterial activity is supported by the polyphenolic content rich in gentisic acid, catechin and epicatechin (López et al., 2015).

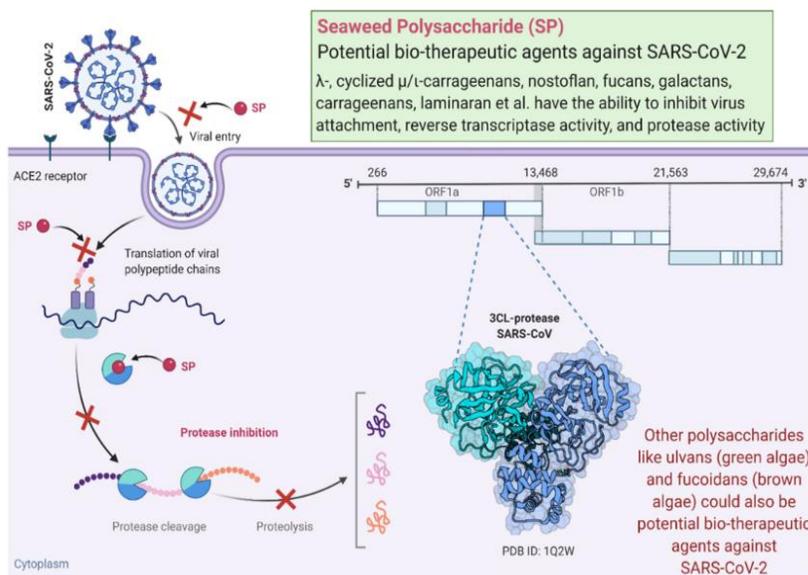
A new approach is represented by the antimicrobial peptides due to their great potential conferred by their structure and functional tuning. Peptide fractions of the pioneering alga *Chlorella vulgaris* were reported by Sedighi et al. to exhibit antibacterial effect (Sedighi et al., 2019). Hydrolysates of 62 kDa were 8.5 and 1.6 times more efficient when compared to *Chlorella* biomass against *Escherichia coli*. In addition, *Chlorella sorokiniana* showed antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus*, as pepsin hydrolysates and peptide fractions (Tejano et al., 2019). Guzmán et al. (2019) reported antibacterial peptides derived from marine microalga

*Tetraselmis suecica* active against both Gram-positive and Gram-negative strains (Guzmán et al., 2019).

New avenues to be explored are linked to the antibiofilm activity of the algal peptides. There are already few reports on cyclic peptides portoamides for instance obtained from *Phormidium* sp. that proved to be active on different marine bacteria by inhibiting ATP-ase H<sup>+</sup>-transport activity (Antunes et al., 2019). This type of property has a direct application in the development of novel antifouling agents.

Marine organisms are rich sources of polysaccharides, and their antiviral activities were first reported over 50 years ago. Modified chitosan is a polysaccharide that significantly inhibits the human coronaviruses HCoV-229E, HCoV-OC43, HCoV-NL63, and HCoV-HKU1. Chitosan is an effective inhibitor of all low-pathogenic human coronaviruses (Pereira & Critchley, 2020; Wang et al., 2012).

Seaweed polysaccharides (SP) can inhibit the life cycle of a virus at different stages via direct inactivating virions before viral infection. Antiviral studies on polysaccharides such as carrageenan and chitosan have exhibited direct virucidal actions on some enveloped viruses to block viral infection. Figure 12 illustrates SP to be notable biotherapeutic agents against SARS-CoV-2 (Alam et al., 2021). Dieckol is a phlorotannin that has been extracted from a brown alga *Ecklonia cava* and is reported as the most potent SARS-CoV 3CLpro trans-/cis-cleavage inhibitory activity in a dose-dependent and competitive manner with no toxicity (Park et al., 2013).



**Figure 12:** A Schematic of Seaweed Polysaccharides (SP) Used As Notable Biotherapeutic Agents against SARS-CoV-2 (Alam et al., 2021)

### 2.2.5. Anti-Diabetic Properties

Diabetes is a metabolic dysfunction marked by chronic hyperglycemia caused by a series of genetic, environmental and nutritional patterns. Clinical manifestation of diabetes includes besides hyperglycemia a high risk of chronic complications linked to interdependent mechanisms among hyperglycemia, insulin resistance, low-grade inflammation and atherogenesis (Sanchez et al., 2017). Western diet, rich in saturated fatty acids and poor in fiber is associated with a high risk of chronic diseases such as diabetes while increased consumption of carbohydrates and protein amplify the risk for diabetes (Malik et al., 2016). Due to their technological and nutritional characteristics, algae are promising candidates as functional foods, with potential for

treatment of diabetes. Algal nutrients and bioactive compounds exhibit functional potential in terms of glycemic control, activation of antioxidant enzymes or reduction of proinflammatory cytokines production (Alkhatib et al., 2017). There is a new hot research topic, nutrigenomics that addresses the modulation of nutrients and bioactive components on gene expression. Furthermore, the diet can regulate epigenome leading to persistent changes in gene expression and also metagenomics by microbiota modulation (Pena-Romero et al., 2018). Recently it was shown that gut microbiota directly interferes with glucose and lipid metabolisms as well as the equilibrium among pro- and anti-inflammatory effectors in the liver with direct consequences on diabetes (Kolodziejczyk et al., 2019). Among the most studied algal compounds with effects on glucose homeostasis are the polyphenols and the fibers. For instance, algal extracts of *Ascophyllum nodosum* and *Fucus vesiculosus* were correlated with insulin regulation (Paradis et al., 2011) while *Undaria pinnatifida* with a reduction of insulin and postprandial glucose levels (Yoshinaga & Mitamura, 2019). Alginate was shown to suppress satiety with dependence on the vehicle used for supplementation. For example, Tanemura established that extracts of *Undaria pinnatifida* in combination with white rice for breakfast reduced postprandial glucose (Tanemura et al., 2014). Similar results were obtained by Yoshinaga & Mitamura, (2019), for Wakame consumption, this type of hypoglycemic effects being linked to reduced risk for diabetes. Kim et al. (2014) selected *Ascophyllum nodosum* as a better candidate for fucoidan source with potential in inhibiting  $\alpha$ -amylase and  $\alpha$ -glucosidase (enzymes involved in increasing

postprandial blood glucose) when compared to *Fucus vesiculosus* (Kim et al., 2014; Naveen et al., 2021) reported the inhibitory activity of *Padina tetrastromatica* alcoholic extracts against  $\alpha$ -amylase ( $IC_{50}$   $47.2 \pm 2.9 \mu\text{g}$ ) and  $\alpha$ -glucosidase ( $IC_{50}$   $28.8 \pm 2.3 \mu\text{g}$ ) due to their polyphenolic content.

Yang et al. (2017) studied *Laminarin* properties on energy homeostasis in mice and found that it can be used in obesity treatment and glucose homeostasis due to a decreased body weight in high fat diet as well as reduced blood glucose (Yang et al., 2017). Further on, the significant results obtained with laminarin are encouraging as it leads to reduced systolic blood pressure, cholesterol absorption in the gut and cholesterol in blood flow.

It is important to assess and determine the algal source, time of harvest and doses treatment as these conditions could condition the beneficial effects.

### **2.2.6. Cosmeceutics Production**

Skin, the largest organ of human body is constantly exposed to various pro-oxidants from the environment, such as solar radiation, pollution, drug consumption, synthetic cosmetic products which are capable to produce ROS at the epidermal level. ROS are prevalent in nature and are permanently produced in aerobic systems. Living organisms are capable to synthesize a wide range of anti-oxidants in order to maintain homeostasis. Main targets to induce toxicity used by ROS are DNA, proteins and lipid-rich membranes. Damages induced at these levels can

lead to diverse skin diseases ranging from atopic dermatitis to vitiligo. Therefore, the discovery of novel natural products that could limit ROS production and their deleterious effects could bring new insight for biomedical and cosmeceutical industry. Various effects are associated to algal metabolites such as photoprotection, skin whitening, antioxidant, antiacne, antifungal and antiallergy, recommending it for their cosmeceutical potential.

*In vitro* antioxidant activity of *Pavlova lutheri* microalga fermented with the yeast *Hansenula polymorpha* was reported by Quian et al. (2012). Tetrapeptide MGRY (MW= 526) reduced the oxidative stress and expressed inhibitory effects in melanogenesis process when evaluated in B16F10 melanoma cells.

The evolution of the skin care industry has been considerably shaped by emergent natural formulations. Marine ingredients and more specifically algal compounds have increased from 6% of the 190 anti-aging cosmetics in 2011 to 33% of the new 103 studied an-aging products in 2018 (Resende et al., 2021). Carotenoids such as astaxanthin are important antioxidants as they play an important role in scavenging free radicals with a considerable presence in the human diet. Top cosmeceutical companies, such as L'Oréal, Henkel, Unilever and Beiersdorf are expected to enhance the growth of carotenoid market value in the European market (Ambati et al., 2019).

Phycocyanin, derived from red algae is used as natural color additive in foods and cosmetics and accepted by the Food and Drug Administration (FDA) due to its non-toxicity and biodegradability characteristics.

### **3. ALGAE AS A SOURCE OF BIOSTIMULANTS**

One of the most innovative applications of macroalgal extracts is biostimulant production. Some researchers even consider macroalgae as the most important group of known organisms that can potentially be used in this industry.

The word biostimulant was apparently coined by horticulture specialists for describing substances promoting plant growth without being nutrients, soil improvers, or pesticides. Tracing back the first definition of the word biostimulants identifies a web journal dedicated to turf maintenance professionals, called Ground Maintenance (<http://grounds-mag.com>). In this web journal in 1997, Zhang and Schmidt from the Department of Crop and Soil Environmental Sciences of the Virginia Polytechnic Institute and State University defined biostimulants as ‘materials that, in minute quantities, promote plant growth’. By using the words ‘minute quantities’ for describing biostimulants, the authors aimed at distinguishing biostimulants from nutrients and soil amendments, which also promote plant growth but are applied in larger quantities.

Biostimulants are organic additives that stimulate plant growth, mainly due to their high content in phytohormones or analogues. Among the main phytohormones that were identified in algal extracts are auxins

(supporting cell division, plant organ elongation and aging), cytokinins (hormones regulating cell division, branching and lateral growth, enhancing mineral nutrient uptake and translocation and limiting tissular aging, chloroplast stimulation), gibberellins (stimulating flower bud opening, flower and fruit maturation and seed germination), ethylene and abscisic acid (accelerating tissular aging and regulating germination; Tuhy et al., 2013).

Depending on the bioactive principle and extraction efficiency, various methods are used in producing bistimulant extracts. Alkaline extraction, for instance, is used for producing auxine-rich extracts. For fucoidan, high-pressure water extraction, combined with microwave assisted extraction, is used. Cold extraction in ethanol is used for gibberellins (85% ethanol) and cytokinins (70% ethanol). Supercritical CO<sub>2</sub> extraction is also becoming widespread in this field, due to its potential to mobilize other key compounds, such as antioxidants and vitamins. Apart from phytohormones, macroalgal extracts contain valuable amounts of fatty acids, steroids, amino acids and polyphenolic compounds that stimulate growth, vigor and resistance to various pathogens. Another key component is represented by polysaccharides. These usually form 30 - 40% of extract dry matter (Tuhy et al., 2013; Kałużewicz et al., 2017).

Macroelements (Ca, K, Mg, Na) and microelements (B, Cd, Co, Cr, Cu, I, Fe, Mo, Mn, Ni, Pb, Sr, Zn) that are often present in large amounts and in soluble forms in algal extracts are another explanation of their potency as plant growth promoters (Duarte et al., 2018).

Marine algae are also rich in osmoprotective compounds, especially 3-dimethylsulfoniopropionate (DMSP), betaines and derivatives. These can help crop plants subjected to saline stress conditions restore normal features such as leaf area and chlorophyll content (El-Beltagi et al., 2019). Furthermore, the same principles also apply to enhancing freeze resistance, an effect documented in thale cress (*Arabidopsis thaliana*) and barley crops (Ali et al., 2021). Algal biostimulants can be applied onto roots, seeds or delivered via foliar spraying (Tuhy et al., 2013).

### **3.1. Main Categories of Plant Biostimulants**

#### **3.1.1 Humic and Fulvic Acids**

Humic substances (HS) are natural constituents of the soil organic matter, resulting from the decomposition of plant, animal and microbial residues, but also from the metabolic activity of soil microbes using these substrates. HS are collections of heterogeneous compounds, originally categorized according to their molecular weights and solubility into humins, humic acids and fulvic acids.

Any attempt to use humic substances for promoting plant growth and crop yield needs to optimize these interactions to achieve the expected outputs. This explains why the application of humic substances – soluble humic and fulvic acids fractions – shows inconsistent, yet globally positive, results on plant growth.

Humic substances have been recognized for long as essential contributors to soil fertility, acting on physical, physico-chemical, chemical and biological properties of the soil (Rose et al., 2014). Most

biostimulant effects of HS refer to the amelioration of root nutrition, via different mechanisms. One of them is the increased uptake of macro- and micronutrients, due to the increased cation exchange capacity of the soil containing the polyanionic HS, and to the increased availability of phosphorus by HS interfering with calcium phosphate precipitation.

Hormonal effects are also described, but whether HS contain functional groups recognized by the reception/signalling complexes of plant hormonal pathways, liberate entrapped hormonal compounds, or stimulate hormone-producing microorganisms is often unclear (du Jardin, 2012).

### **3.1.2. Protein Hydrolysates and Other N-Containing Compounds**

Amino-acids and peptides mixtures are obtained by chemical and enzymatic protein hydrolysis from agroindustrial by-products, from both plant sources (crop residues) and animal wastes (e.g. collagen, epithelial tissues) (du Jardin, 2012; Calvo et al., 2014; Halpern et al., 2015).

Case by case, these compounds have been shown to play multiple roles as biostimulants of plant growth (du Jardin, 2012; Calvo et al., 2014; Halpern et al., 2015). Direct effects on plants include modulation of N uptake and assimilation, by the regulation of enzymes involved in N assimilation and of their structural genes and by acting on the signalling pathway of N acquisition in roots. By regulating enzymes of the TCA cycle, they also contribute to the cross talk between C and N metabolisms.

Several commercial products obtained from protein hydrolysates of plant and animal origins have been placed on the market. Variable, but in many cases significant improvements in yield and quality traits have been reported in agricultural and horticultural crops (Calvo et al., 2014). Nevertheless, there is a growing safety concern of using protein hydrolysates derived from animal by-products in the food chain. The EU banned the application of such animal protein hydrolysates on the edible parts of organic crops, through the Commission Implementing Regulation (EU) no 354/2014 with regard to organic production, labelling and control.

### **3.1.3. Seaweed (Marine Algae) Extracts and Botanicals**

The use of fresh seaweeds as source of organic matter and as fertiliser is ancient in agriculture, but biostimulant effects have been recorded only recently. This prompts the commercial use of seaweed extracts and of purified compounds, which include the polysaccharides laminarin, alginates and carrageenans and their breakdown products. Other constituents contributing to the plantgrowth promotion include micro- and macronutrients, sterols, *N*-containing compounds like betaines, and hormones (Khan et al., 2009; Craigie J.S., 2011). Several of these compounds are indeed unique to their algal source, explaining the increasing interest of the scientific community and of the industry for these taxonomic groups. Most of the algal species belong to the phylum of brown algae – with *Ascophyllum*, *Fucus*, *Laminaria* as main genera, but carrageenans originate from red seaweeds, which correspond to a distinct phylogenetic line.

Seaweeds act on soils and on plants (Craigie, 2011; Khan et al., 2009). They can be applied on soils, in hydroponic solutions or as foliar treatments. In soils, their polysaccharides contribute to gel formation, water retention and soil aeration. The polyanionic compounds contribute to the fixation and exchange of cations, which is also of interest for the fixation of heavy metals and for soil remediation. Positive effects via the soil microflora are also described, with the promotion of plant growth-promoting bacteria and pathogen antagonists in suppressive soils. In plants, nutritional effects via the provision of micro- and macronutrients indicate that they act as fertilisers, beside their other roles (Hamed et al., 2018). Impacts on seed germination, plant establishment and on further growth and development is associated with hormonal effects, which is viewed as major causes of biostimulation activity on crop plants. Although cytokinins, auxins, abscisic acid, gibberellins and other classes of hormone-like compounds, like sterols and polyamines, have been identified in seaweed extracts by bioassays and by immunological tools (Craigie, 2011), there is evidence that the hormonal effects of extracts of the brown seaweed *Ascophyllum nodosum* are explained to a large extent by the down- and upregulation of hormone biosynthetic genes in plant tissues, and to a lesser extent to the hormonal contents of the seaweed extracts themselves (Wally et al., 2013 a,b).

Among green macroalgae, important sources of biostimulants are species like *Cladophora dalmatica*, *Codium taylorii*, *Ulva intestinalis*, *Ulva fasciata*, *Ulva lactuca* (Tuhy et al., 2013).

*Codium taylorii* and especially *Ulva lactuca* and *Ulva fasciata* water filtrates were found to be particularly effective at enhancing seed germination and seedling growth in pea plants (*Pisum sativum*), with up to 33% extra growth compared to control plants and 27% compared to plants treated with commercial hormone mixtures (Duarte et al., 2018). *Ulva* extracts also reduce germination time in tomato plants (Pérez-Madruga et al., 2020).

*Ulva lactuca* extracts were successfully used to restore wheat growth under high salinity (El-Beltagi et al., 2019). *Ulva* extracts were also found to alleviate oxydative stress usually associated with osmotic stress, probably due to its high polyphenolic content. Up to 8% increases in shoot length and 27% increases in biomass were observed in wheat plants treated with such extracts (Latique et al., 2021).

Some red algae are also known for their biostimulant properties, such as *Corralina mediterranea*, *Fucus vesiculosus*, *Gelidium crinale*, *Gracilaria caudata*, *Gracilaria cervicornis*, *Gracilaria edulis*, *Jania rubens*, *Kappaphycus alvarezii*, *Laminaria digitata*, *Laminaria hyperborea*, *Padina durvillaei*, *Padina gymnospora*, *Palisada peforata*, *Pterocladia pinnata* (Tuhy et al., 2013; Duarte et al., 2018).

On experiments run on pea plants, *Gracilaria caudata*, *Palisada peforata* and *Padina gymnospora* extracts were proven to be the most effective in enhancing seedling elongation, with up to 16%, in comparison to commercial phytohormones (Duarte et al., 2018). 15% extracts of *Gracilaria* and *Kappaphycus*, combined with traditional fertilizers were shown to enhance nitrogen uptake and metabolism in

rice, increasing the number of panicles, panicle length, grain and straw yield. 10% extracts of the same species, sprayed onto plant leaves led to 42-47% increases in grain yield in black gram (*Vigna mungo*) and increased the ratio of marketable to non-marketable tubers and lowered tuber damage in potato crops (Al-Juthery et al., 2020). 7.5% *Kappaphycus alvarezii* extracts increased general yield in both maize and potatoes (El Boukhari et al., 2020).

*Gelidium crinale* extracts are known to help alleviate the effects of osmotic stress in canola, causing an increase in stem length, number of fruits and seeds and seed weight (Pérez-Madruga et al., 2020). The same was found in *Grateloupia filicina* extracts applied in rice crops (El Boukhari et al., 2020).

*Padina* extracts were also used as insecticides, increasing mortality in nymphs and lowering fertility (Pérez-Madruga et al., 2020).

Among brown algae, biostimulant properties were found in *Ascophyllum nodosum*, *Cystoseira* spp., *Durvillaea potatorum*, *Ecklonia maxima*, *Saragassum* spp., *Turbinaria* spp. (Tuhy et al., 2013; Pérez-Madruga et al., 2020).

For instance, *Ascophyllum nodosum* is a good source of growth-promoting phytohormones and polysaccharides. Experiments on broccoli (*Brassica oleracea* var. *italica*) cultures showed that *Ascophyllum nodosum* filtrate application, in conjunction with an amino acid-based biostimulant, had a positive effect on polyphenolic

synthesis in plant organs (especially on caffeic acid) and, thus, on the quality of the final product (Kałużewicz et al., 2017).

*Ascophyllum nodosum* extracts are known to support nitrogen and boron fertilization on fruit trees and olive trees. Foliar application led to growth stimulation, larger fruits and, respectively, a higher quality of olive oil (Tuhy et al., 2013). In wheat, supercritical CO<sub>2</sub> extract spraying of *Ascophyllum nodosum* was proven to have positive effects on spike length, grain numbers per spike and grain mass (however, not translated into a superior total grain yield/ha). Interesting enough, obtained values were higher than those obtained with commercial growth stimulants containing amounts of *Ascophyllum nodosum* extracts, supplemented with minerals (Michalak et al., 2016).

*Ascophyllum nodosum* is also rich in betaines, helping alleviate osmotic stress in plants. Betaines can also work as nitrogen source, under certain conditions. Extracts of this alga (and also of *Sargassum vulgare*) were successfully used to increase salinity tolerance and stimulate flowering under osmotic stress in amaranth (*Amaranthus tricolor*) and to stimulate germination and seedling growth on saline soils in tomato. Osmoprotective compounds also work increasing plant tolerance to water deficit, as was proven on bean plants by using *Durvillaea potatorum* extracts (El-Beltagi et al., 2019).

*Ascophyllum* extracts also have an indirect effect on plant growth, stimulating local beneficial microbial activity, which, in turn, stimulates root growth. The same species can directly or indirectly (through stimulating competing microbiota) prevent infections with pathogens

like *Phytophthora capsici* (in pepper), *Plasmopara viticola* (in grapevine) or *Pythium ultimum* (in cabbage; Pérez-Madruga et al., 2020).

Finally, *Ascophyllum*, together with *Ecklonia maxima* can successfully supplement NPK fertilizers and humic acid in forage grass, turf grass and flower crops. They stimulate stem growth, leaf area, vegetative reproduction, chlorophyll, carotenoid and antioxidant contents, resistance to heat and drought-induced stress and transplantation-related stress (Battacharyya et al., 2015).

*Sargassum horneri* extract was tested on tomato crops. At 60-90 kg/ha spraying, it improved overall photosynthetic activity, leading to 4.6-6.9 yield increases and lowering losses due to storage and transport, by hardening fruit tissues (Al-Juthery et al., 2020).

*Sargassum vulgare*, together with the red alga *Kappaphycus alvarezii* is used for the production of a commercial biofertilizant, rich in B, Fe, Mg and S, amino acids and phytohormones. Applied on pepper plants (*Capsicum annuum*), at 0.5-2% concentrations, it led to increases in leaf number (8-148%), root length (123-230%), dry biomass (2-113%), total fruit mass (32-69%; Melo et al., 2020).

*Ecklonia maxima* extracts are rich in auxins and cytokinins. Applied on wheat crops (2-3 L/ha, as early or late treatments or combined), they led to increases in some grain yield (up to 5%), however, correlated with decreases in straw yield (Szczepanek et al., 2018). Sprayed on soybean leaves (0.250-1 L/ha), they stimulated stem branching and a

higher number of flowers and pods, with an overall 6% yield increase (Meyer et al., 2020).

Even giant algae, like *Lessonia flavicans*, *Lessonia nigrescens* and *Macrocystis pyrifera* were proven to have an indirect biostimulant effect, through stimulating enzyme-producing bacteria and increasing the hydrogenase, invertase, phosphatase, proteinase and urease activity in soil in tea crabapple (*Malus hupehensis*) orchards (El Boukhari et al., 2020).

‘Botanicals’ describe substances extracted from plants which are used in pharmaceutical and cosmetic products, as food ingredients, and also in plant protection products (Seiber et al., 2014). Compared with seaweeds, much less is known regarding their biostimulant activities, the attention being focused on their pesticidal properties so far. However, there seems to be opportunities to use them as biostimulants as well (Ertani et al., 2013; Ziosi et al., 2012).

#### **3.1.4. Chitosan and Other Biopolymers**

Chitosan is a deacetylated form of the biopolymer chitin, produced naturally and industrially. Poly- and oligomers of variable, controlled sizes are used in the food, cosmetic, medical, agricultural and horticultural sectors. The physiological effects of chitosan oligomers in plants are the results of the capacity of this polycationic compound to bind a wide range of cellular components, including DNA, plasma membrane and cell wall constituents, but also to bind specific receptors involved in defense gene activation, in a similar way as plant defense

elicitors (El Hadrami et al., 2010; Yin et al., 2010; Hadwiger, 2013; Katiyar et al., 2015).

Agricultural applications of chitosan have been developed over the years, focusing on plant protection against fungal pathogens, but broader agricultural uses bear on tolerance to abiotic stress (drought, salinity, cold stress) and on quality traits related to primary and secondary metabolisms.

Several poly- and oligomers of biological origin or (hemi-) synthetic variants are increasingly used in agriculture as elicitors of plant defense, including seaweed polysaccharides which we have already mentioned. A good example is laminarin, a storage glucan of brown algae, of which purified preparations are used in agricultural and horticultural applications.

### **3.1.5. Inorganic Compounds**

Chemical elements that promote plant growth and may be essential to particular taxa but are not required by all plants are called beneficial elements (Pilon-Smits et al., 2009). The five main beneficial elements are Al, Co, Na, Se and Si, present in soils and in plants as different inorganic salts and as insoluble forms like amorphous silica ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ) in graminaceous species. These beneficial functions can be constitutive, like the strengthening of cell walls by silica deposits, or expressed in defined environmental conditions, like pathogen attack for selenium and osmotic stress for sodium.

Their function as biostimulant of plant growth, acting on nutrition efficiency and abiotic stress tolerance, hence distinct from their fungicidal action and from their fertiliser function as sources of nutrients, deserves more attention.

### **3.1.6. Beneficial Bacteria**

Bacteria interact with plants in all possible ways (Ahmad et al., 2008):

- (a) As for fungi there is a continuum between mutualism and parasitism;
- (b) Bacterial niches extend from the soil to the interior of cells, with intermediate locations called the rhizosphere and the rhizoplane;
- (c) Associations may be transient or permanent, some bacteria being even vertically transmitted via the seed;
- (d) Functions influencing plant life cover participation to the biogeochemical cycles, supply of nutrients, and increase in nutrient use efficiency, induction of disease resistance, enhancement of abiotic stress tolerance, modulation of morphogenesis by plant growth regulators.

A common designation of biostimulants is only justified if the described substances and microorganisms share some important characteristics regarding their natures, functions and/or uses. Such characteristics would then be the ground for any definition. From the bibliographic review, the following conclusions may be drawn:

- The nature of biostimulants is diverse. Substances and microorganisms are involved. Substances can be single compounds (e.g.

glycine betaine) or groups of compounds of single natural origin of which the composition and bioactive components are not fully characterized (e.g. seaweed extracts); the substances commented by this review are naturally produced organic compounds, or inorganic molecules, but synthetic compounds should not be excluded, especially if certain plant growth regulators are included within biostimulants (for example, nitrophenolates are described and commercialized as 'biostimulants' but are synthetic phenolic compounds registered as plant production products according to the EU Law, see Przybysz et al. (2014).

- The physiological functions are diverse. By physiological function, we refer to any action on plant processes. Examples of physiological functions are the protection of photosynthetic machinery against photo damage, or the initiation of lateral roots.
- The scientifically demonstrated effects of all biostimulants converge to at least one or several of the following agricultural functions: they enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits.
- Definition of economic and environmental benefits depends on agri-cultural and environmental policies, both in terms of objectives and assessment endpoints.

In conclusion any definition of biostimulants should focus on the agricultural and horticultural functions of biostimulants, not on the nature of their constituents nor on their modes of actions, as they have been defined above.

## **4. MARINE ALGAE AS BIOFERTILIZERS IN AGRICULTURE AND HORTICULTURE**

Algae play an important role in agriculture where they are used as biofertilizers and soil stabilizers (Negreanu-Pirjol et al., 2017). Algae, particularly the seaweeds, are used as fertilizers, resulting in less nitrogen and phosphorous runoff than the one from the use of livestock manure.

The presence of algae leads to reduced erosion by regulating the water flow into soils. Similarly, they play a role in soil reclamation, bio-controlling of agricultural pests, formation of microbiological crust, agricultural wastewater treatment and recycling of treated water (Negreanu-Pirjol et al., 2012; Vatafu et al., 2015). Human civilization depends on agriculture for its existence. The success of agriculture greatly depends on the fertility level of the soil. Like other organisms, algae which are found in different soil types may help the soil to improve its characteristics such as, carbon content, texture, aeration and also nitrogen fixation. The magnitude of these improvements is greatly dependent on the physical and chemical characteristics of the soil, affecting the composition of the algal population. Some big reasons why we need to start use more algae in agriculture and horticulture are (Negreanu-Pirjol T. et al., 2011; Cai et al., 2021).

### **4.1. Improvement of Soil Fertility**

Nitrogen to ammonia, a process where oxygen evolved by photosynthetic activity in the same cell is detrimental to nitrogen fixation (Mus et al., 2016). Strategies to avoid oxygen range from the

temporal separation of nitrogen fixation and oxygen evolution (in many unicellular and filamentous, nonheterocysts strains) to spatial separation and cellular differentiation into nitrogen-fixing heterocysts (in *Filamentous cyanobacteria*) (Fay, 1992). Heterocysts are terminally differentiated cells whose interior becomes anaerobic, mainly as a consequence of respiration, allowing the oxygen-sensitive process of nitrogen fixation to continue (Wagner, 2011).

#### **4.2. Uptake of P and N**

Phosphorus (P) is the second important nutrient after nitrogen for plants and microorganisms (Steinman & Duhamel, 2017). Most aquatic systems are resource-limited, where P and N are often the primary limiting nutrients (O'Hara et al., 2002; Abdel-Raouf et al., 2012). To ensure survival, a competitor must be able to maintain net population growth at resource levels less than those required by other species. Algae are particularly adapted to scavenge their environments for resources through structural changes, storage, or increased resource utilization efficiency (Chojnacka et al., 2012). Internal adjustments by algae involve biochemical and physiological adaptations, whilst they can also excrete substances to enhance nutrient availability. Algae excrete extracellular phosphatases almost immediately upon the onset of P-limited conditions (Abdel-Raouf et al., 2012; Vrba et al., 2018).

#### **4.3. Source of Organic Matter**

Algae are also an important source of organic matter in the soil (Zodape, 2001). The organic matter formed from the death and decay of algae

may get mixed in the soil and mucilage acts as a binding agent for soil texture, thereby increasing the humus content and making it more habitable for other plants after some years. Humus accumulation is also important for moisture retention (Abdel-Raouf et al., 2012).

#### **4.4. Soil Reclamation**

The difficulties in soil reclamation in arid and semi-arid regions are mostly the salinity conditions of large soil areas and the effect of salinity on the growth, metabolism and yield of plants and algae (Nabti et al., 2010). Some growth regulators such as gibberellic acid ( $GA_3$ ) were used for improving the salt tolerance of the plants (Jennings, 1968; Rayorath et al., 2008). From an economic point of view, growth regulators are expensive and are non-practical especially, when applied in large amounts. Algae play an economic role in soil reclamation increases soil fertility and improve plant conditions under certain environmental factors (Raimi et al., 2017).

#### **4.5. Biological Control**

Biological control means the replacement of chemical pesticides by natural components of different plant and microalgal sources as insecticide agents, acaricide agents and fungicidal agents (Chowdhury et al., 2015). These natural materials in addition to their lethal activities on pests preserve the environment of pollution, maintain the equal distribution of fauna, and also keep the beneficial animals (Abdel-Raouf et al., 2012). Fungi and bacteria are the main biological agents

that have been studied for the control of plant pathogens, particularly soil-borne fungi (Kulik, 1995).

One of the modern and advanced biotechnological researches is that conducted on the using of different algal taxa of different habitats (marine, fresh and soil) as a biological control for many animal or plant diseases and also against agricultural pests. Some of these researches studied the antimicrobial activities against some human pathogenic bacteria, fungi and toxic micro-algae (Alves et al., 2016; Watee et al., 2015; Pérez et al., 2016). Others were conducted on the study of toxic effects of some algal metabolites against insects (Schrader et al., 2002).

## **CONCLUSIONS**

Algae are important components of arid and semi-arid ecosystems. Furthermore, their distribution may indicate the health of the environment. In recent years, many considerations were sent towards the possibility of using algae as biological conditioners instead of any artificial or chemical conditioners, where algal use reduces the resultant pollution to soil and plants together, in addition to their ability to improve both soil and plant properties.

Marine algae, microalgae and cyanobacteria are ubiquitous in the world's soils. Although they are the primary microbial photosynthetic agents of the soil, their ecological role is still not fully defined. In this study, emphasis was laid on the role of algae, in soil fertility and reclamation and some of their advantageous properties and beneficial effects influence the plant/soil system, such as:

- Excretion of organic acids that increase P-availability and P-uptake,
- Provision of nitrogen by biological nitrogen fixation,
- Increased soil organic matter,
- Production and release of bioactive extracellular substances that may influence plant growth and development. These have been reported to be plant growth regulators (PGRs), vitamins, amino acids, polypeptides, antibacterial or antifungal substances that exert phytopathogen biocontrol and polymers, especially exopolysaccharides, that improve soil structure and exoenzyme activity.
- Stabilization soil aggregation by extracellular polysaccharides of soil aggregate
- Concentrate metal ions present in their environment.

Agricultural and horticultural use of biostimulants will require locally and temporally adapted solutions. Monitoring tools for the efficacy of biostimulants will be needed and stewardship plans optimising their use defined. Available information on the potential roles of marine macroalgae in plant protection and improvement is still poorly known so far. In general, marine macroalgae are mainly characterized by the presence of particular components of a biotechnological interest in integrated pest management such as microbicides, nematicides, insecticides, biofertilizers, biostimulators and soil conditioners. All these substances are considered ecofriendly safe to the environment for organic farming practices. However, many deep investigations on this

trend of study are still needed to discover novel substances. Finally, marine macroalgae and their extracts could provide a chance for increasing percentage of plant cultivation in harsh habitats and are important bioinoculants in recent trends of organic farming for achieving sustainable agriculture and horticulture development (Hamed et al, 2018).

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**CHAPTER VII**

**PHYSICOCHEMICAL CHARACTERISTICS AND  
BIOLOGICAL ACTIVITIES OF MATURE DEGLET-NOUR  
DATE FRUIT**

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## 1. INTRODUCTION

The date (*Phoenix dactylifera* L.) is an exceptional fruit due to its high nutritional value and its organoleptic properties, making it a fundamental food for the Saharan populations. Consumed fresh or dry, whole or transformed into by-products; the date has always been used in the traditional medication of these populations. Its therapeutic virtues and pharmacological properties are subjects of several research works around the world (Baliga et al., 2011; Hussain et al., 2020). Dates contain high amounts of carbohydrates and dietary fibres besides important contents of vitamins, minerals and phenolic compounds. On the other hand, dates contain a higher percentage of protein than other type of fruits, also, important fatty acids and carotenoids (Al-Shahib & Marshall, 2003). Phenolic are important bioactive compounds of plants which have multiple health benefits. They are currently of growing research interest so that foods rich in these compounds are considered to be functional.

It is in this context that the present study was carried out in order to highlight some physicochemical characteristics and biological activities of Deglet-Nour date, one of excellence date varieties, highly appreciated throughout the world. The possible involvement of the fruit's phenolic compounds in its biological activities was also aimed by the study.

As plants are useful and important sources to find new bioactive agents, antibacterial and haemostatic ones were sought in fruit extracts by evaluating correspondent activities.

Antioxidant activity was more targeted via several methods because oxidative stress is a factor in inflammation and mutagenesis (Aoki et al., 2020). Besides being considered one of the main causes of cancer (Jelic et al., 2021), it is thought to play a role in several diseases.

Physicochemical characteristics of Deglet-Nour were also targeted to explore the quality parameters of the most succulent and popular cultivar of dates.

## **2. MATERIAL AND METHODS**

### **2.1. Chemicals and Reagents**

Gallic acid, quercetin, catechin, vanillin, caffeic acid, Folin Ciocalteu, aluminium chloride ( $\text{AlCl}_3$ ), sodium carbonate ( $\text{Na}_2\text{CO}_3$ ), ascorbic acid, 2,2-diphenyl-picrylhydrazyl (DPPH),  $\beta$  carotene, linoleic acid, Tween 40, butyl-hydroxy-toluene (BHT), ferrous chloride ( $\text{FeCl}_2$ ) also chloroform, dimethyl-sulfoxide, acetone, acetic acid, petroleum ether, ethyl acetate were all of analytical grade, they were purchased from Sigma Aldrich (St. Louis, MO, USA).

### **2.2. Plant Material**

The date fruit used in the study 'Deglet-Nour' was harvested from a palm grove at the end of October and was sorted and stored in the refrigerator (4 °C) until the time of analysis, in order to slow the chemical and physiological changes. It's of semi-soft consistency and fibrous texture. The choice of this variety was justified by its taste quality, its high production and its availability on the market as well as its wide consumption. Experiences were conducted using fruits of two

consecutive years to ensure replication in time besides samples replication (three per sample for every year).

### **2.3. Bacterial Strains**

*Staphylococcus aureus* ATCC 25923, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* (ESBL), *Bacillus spp*, *Streptococcus agalactiae*. Methicillin-resistant *Staphylococcus aureus* (MRSA), *Acenitobacter sp* and *Entérobacter cancerogenus* ATCC 35316 were bacterial strains used in the study of antibacterial activity of date extracts.

### **2.4. Blood**

Blood has been used to determine haemostatic activity of fruit extracts; it came from healthy people.

### **2.5. Methods**

#### **2.5.1. Morphological Characterization of Whole Fruit**

Morphological characterization of entire date was carried out on a lot sampled randomly. The color was appreciated visually and using a Konica Minolta colorimeter (CR-400, Japan), which was calibrated with a white ceramic plate before each measurement time. Colour was expressed as luminance (L\*), and two colour channels (a\*, and b\*); the weight (pulp and seed) was determined using an analytical balance; the consistency was estimated by touch; the dimensions of the whole fruit and its kernel (length and width) were taken using a calliper.

### **2.5.2. Determination of pH and Water and Ash Contents**

In addition to determination of morphological characteristics of the fruit, pH and water and ash contents were estimated following standard methods. For pH measurement an aqueous macerate of date was used. To determine ash content, destruction of the organic matter was carried out at the temperature of 500 °C for 5 to 6 h using a muffle furnace.

### **2.5.3. Preparation of Fruit Extracts**

A crude extract was prepared at first by solid-liquid extraction using 1200 g of date fruit and 4 L of solvents mixture, acetone / water in the proportions of 60/40 (V/V). Fruit maceration was conducted at room temperature with stirring, then filtration and concentration by evaporation of acetone at 35 °C using rotavapor.

To fractionate the crude extract, the aqueous solution obtained after evaporation of acetone was treated with three organic solvents with increasing polarity, the petroleum ether (2x100 mL), then the chloroform (3x200 mL) and finally the ethyl acetate (3x200 mL). Combined solutions were concentrated under reduced pressure to dryness. Obtained dry extracts have been kept at cold until assays and tests of biological activities.

In addition aqueous crude macerate and syrup of fruit were prepared separately. Major characteristics of extracts were noted for being elucidated in results section. The content of total soluble solids (TSS) among others was measured on aqueous extract and syrup, using a refractometer.

#### **2.5.4. Phytochemical Screening of Extracts**

Several tests were carried out to highlight the presence of certain chemical groups in prepared extracts, which may be responsible for the biological activities studied.

The presence of these groups was tested according to standard methods as described by Dohou et al. (2003) for condensed tannins, Hadj-Salem (2009) for flavonoids; on the other hand, the Fehling test and the Lieberman-Burchard test were used to demonstrate presence of reducing sugar, sterols and triterpenes respectively.

Depending on their intensity, occurring reactions are classified from: negative (-) to totally positive (++++).

#### **2.5.5. HPLC Analysis**

Phenolic compounds were identified using HPLC (Shimadzu model) with an RP-C18 column at 35°C. The extracts were injected in 10 µL volume. The mobile phase was composed of methanol/water (60: 40) (V/V) with isocratic elution. Detection was performed at 254 nm using a spectrophotometer.

#### **2.5.6. Determination of Total Extractable Polyphenol Content**

To a volume of 200 µL of every extract, was added 1 mL of Folin-Ciocalteu reagent (diluted ten times). After 4 min, a volume of 800 µL of sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) (75 mg / mL in distilled water) was added to the preparation. The tubes were placed in the dark for two hours; the optical densities were read at 765 nm.

Total phenol quantification was carried out according to a calibration curve of gallic acid. The results were expressed as milligrams gallic acid equivalents per kilogram of fruit fresh pulp.

#### **2.5.7. Determination of Flavonoid Content**

Aluminium chloride colorimetric method was used for flavonoids content determination. A volume of 1 mL of 2% AlCl<sub>3</sub> methanol solution was added to 1 mL of sample solution. After incubation at room temperature for 30 min, the absorbance was read at 430 nm. Flavonoids content was estimated using a quercetin calibration curve and results were expressed as mg quercetin equivalents per kilogram of fresh pulp.

#### **2.5.8. Determination of Condensed Tannin Content**

For 400 µL of each sample or standard catechin, 3 mL of a solution of 4% vanillin in methanol and 1.5 mL of concentrated hydrochloric acid were added. The mixture was incubated for 15 min and the absorbance was read at 500 nm. Results of condensed tannins contents were expressed as mg catechin equivalents per kilogram of fresh pulp.

#### **2.5.9. Evaluation of Antioxidant Activity**

Study of antioxidant activity of Deglet-Nour extracts was carried out using thin layer chromatography to detect the scavenging activity against DPPH radical, which was also estimated by spectrophotometry. On the other hand, B-carotene bleaching test and antioxidant power of iron reduction test were also used.

### 2.5.9.1. Antiradical Activity Test

Thin layer test against DPPH was carried out using silica gel GF<sub>254</sub> plates; extracts were deposited in spots on silica gel plates and developed in appropriate systems. After migration and drying, the TLCs were sprayed with a methanolic solution of DPPH (2 mg/L). The chromatographic plates were then dried at ambient temperature for few minutes. The visible appearance of yellow spots on a purple background, testifies the presence of antiradical activity.

Estimation of antiradical activity's rates was realized by adding 975 µL DPPH solution to 25 µL of extract or standard solutions (caffeic and ascorbic acids) at different concentrations, the mixture was left in the dark for 30 min and the discoloration compared to the negative control containing only DPPH solution was measured at 517 nm (Mansouri et al., 2005). The DPPH radical inhibition was calculated as percentage according to the following equation:

$$\begin{aligned} & \text{DPPH scavenging activity (\%)} \\ & = \left[ \frac{1 - (\text{absorbance of sample} - \text{absorbance of blank})}{\text{absorbance of control}} \right] * 100 \end{aligned}$$

The necessary amount of samples and standards to decrease the initial DPPH concentration by 50% was determined graphically as (IC<sub>50</sub>) from obtained inhibition percentages and results were thus expressed in antiradical power (ARP) which is the reverse value of IC<sub>50</sub>.

### **2.5.9.2. B-carotene / Linoleic Acid Bleaching Test**

The  $\beta$ -carotene / linoleic acid emulsion was prepared by solubilisation of 0.5 mg of  $\beta$ -carotene in 1 mL of chloroform, 25  $\mu$ L of linoleic acid and 200 mg of Tween 40 were added thereafter. The chloroform was completely evaporated using a rotavapor, 100 mL of oxygenated water were added and the resulting emulsion was stirred vigorously. A volume of 350  $\mu$ L of standard extract or antioxidant solution (BHT) solubilized in methanol (2 mg/mL) was added to 2.5 mL of the preceding emulsion. The kinetics of decolourization of the emulsion in the presence and absence of antioxidant (negative control in which the sample was replaced by 350  $\mu$ L of methanol) was monitored at 490 nm at regular time intervals for 48 hours. The rate of bleaching of  $\beta$ -carotene was calculated as relative antioxidant activity (RAA) using the equation:

$$RAA\% = Ae * \frac{100}{AC}$$

Where Ae is absorbance in the presence of fruit extracts and Ac is positive control's (BHT) absorbance.

### **2.5.9.3. Reducing Power Test**

The ferric reducing power of date extracts was determined according to the method of Oyaizu (1986). In a test tube containing 2.5 mL of sample solution, 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL of Potassium ferricyanide (10 g/L) were added. The mixture was heated at 50 °C in a water bath for 20 minutes. A volume of 2.5 mL of

trichloroacetic acid (100 g/L) was then added and the mixture was centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of the supernatant was mixed with 2.5 mL of distilled water and 0.5 mL of ferric chloride [FeCl<sub>3</sub>] (1 g/L). A blank without sample was prepared under the same conditions. The absorbance was measured at 700 nm. BHT was used as the positive control. Relative reducing power was calculated as: RRP % =  $A_e \cdot 100 / A_c$ , where  $A_e$  is absorbance in the presence of fruit extracts and  $A_c$  is positive control (BHT) absorbance.

#### **2.5.9.4. Determination of Antibacterial Activity**

The antibacterial activity evaluation was carried out by agar disc diffusion method as described by Daas Amiour et al. (2014). From a pure culture of 18 hours on isolation medium nutrient agar, suspensions have been prepared (Inoculums of 0.5 Mc Farland opacity) from each of the bacterial strains to be tested. Whatman discs of 6 mm diameter were impregnated with a small amount of the extracts (20  $\mu$ L per disc) and deposited on the surface of the inoculated Mueller-Hinton agar. Whatman DMSO impregnated paper disc as a negative control was also deposited on the surface of the inoculated agar. Petri dishes were incubated at 37 °C for 24 hours. The reading of antibiograms was done using a caliper. An extract was considered active when a diameter of an inhibition zone around the disk was greater than 6 mm and within which no bacterial growth was observed.

#### **2.5.9.5. Evaluation of Haemostatic Activity**

This test consists of measuring the coagulation time of decalcified plasma after re-calcification (Brummel et al., 2002). The test was performed in vitro on blood plasma from healthy people using aqueous extract and syrup. Blood was collected on sodium citrate and plasma was obtained after centrifugation at 3600 rpm, for 10 min. Distribution of extracts solutions at a rate of: 50, 100 and 200  $\mu\text{L}$  (equivalent of 5, 10 and 20 mg of extract) respectively in test tubes which were kept in water bath at 37 °C and then added of 0.2 mL of plasma and 0.2 mL of 0.025 M calcium chloride. A control sample was also prepared using water instead of extract.

To note exactly the time of coagulation, inclination of each tube at an angle of 45 °C every 30 sec at first, then frequently thereafter was performed, until observation of clot solidification.

#### **2.5.10. Statistical Analysis**

Results were expressed as mean  $\pm$  standard deviation. The differences between samples were determined by analysis of variance (one-way ANOVA) followed by Tukey test. All results were analysed using Graph Pad Prism version 5.01.

### **3. RESULTS AND DISCUSSION**

#### **3.1. Characteristics of the Whole Fruit and Prepared Extracts**

Determined characteristics of studied fruit are expressed in Table 1. The visually determined color of the Deglet-Nour date was brown. In usual

fresh Deglet-Nour has amber yellow color (Hannachi et al., 1998). The brown color may be due to non-enzymatic browning reactions which are accentuated by direct exposure to the sun. This color was confirmed obtaining L\* parameter measure (32.05). Also noting that, amber yellow Deglet-Nour gave 37.31 as mentioned by Dassamiour et al. (2018). Semi-soft consistency of studied Deglet-Nour has been consolidated by the evaluation of its water content (25.67%). indeed a soft consistency of this variety of date coincides with water contents going up to 28-29%. Note that water content of dates is closely linked to the humidity of the external environment; therefore this value varies from one region to another and even from one microclimate to another. The weight and color of the date reflect its good quality. Deglet-Nour date is tapered or ovoid in shape. It is composed of a big seed, having a hard consistency, surrounded by flesh. Measurements taken of the weight and dimensions of the whole fruit as well as its constituents (pulp and seed) indicate that it is of good quality compared to the same characteristics reported by Hannachi et al. (1998) and Belguedj (2002). On the other hand, the pH value and the ash content found in this study also indicate the good quality of the fruit studied.

**Table 1:** Characteristics of Studied Deglet-Nour Fruit

Parameters	Means values
Color	Light brown
L*	32.05 ± 0.41
a*	6.24 ±0.60
b*	7.40 ±0.86
Consistency	Semi-soft
Weight of whole date(g)	10.15± 0.64

Poids de la pulp (g)	8.27 ± 0.61
Weight of seed (g)	0.98± 0.11
Date length (cm)	4.56 ± 0.20
Seed length (cm)	1.96 ± 0.05
Width (diameter) of whole date (cm)	1.78 ± 0.09
Seed width (diameter) (cm)	0.75 ± 0.05
Pulp / whole date ratio (%)	81.47 ± 0.30
Seed / whole date ratio (%)	9.65 ± 0.10
pH	5.45 ± 0.25
Water content (% of fresh matter)	25.67 ± 1.31
Ash content (% of dry matter)	2.00± 0.57

Values were expressed as means ± SD (n = 3).

In fact, the pH of the same cultivar, coming from the same palm grove, measured within 48 hours after harvest was around 5.73 (Dassamiour et al., 2018); a slightly higher value.

The 2% ash content indicates that Deglet-Nour contains a significant amount of mineral matter. Indeed, it is well established today that the date contains sodium, potassium, calcium, magnesium, phosphorus, copper, iron, zinc, manganese and selenium (Baliga et al., 2011).

Fractionation of crude extract obtained after evaporation of acetone was carried out by solvents of increasing polarity which allowed obtaining petroleum ether extract (Ep E), chloroform extract (Ch E) and ethyl acetate extract (EA E). The colour and appearance as well as the yield of each of these extracts, in addition to content in total soluble solids (TSS) of aqueous macerate and syrup are given in Table 2.

**Table 2:** Characteristics of Deglet-Nour Extracts

<b>Extract</b>	<b>Yield (%)</b>	<b>Color</b>	<b>Aspect</b>	<b>TSS (°Brix)</b>
<b>PE E</b>	1.47	Dark brown	Oily	-
<b>Ch E</b>	2.58	Light brown	Viscous	-
<b>EA E</b>	1.10	Yellow	Pasty	-
<b>Aq E</b>	32.89	Brown	Viscous	30
<b>Syrup</b>	45.15	Dark brown	Viscous	76

Aqueous extract and syrup gave the highest yields; this result was expected because the date is very rich in polar compounds, especially carbohydrates. In addition, Deglet-Nour contains more simple sugars, easily extractable, than other semi-soft varieties.

### **3.2. Characterised Chemical Groups and Contents of Total Phenols, Condensed Tannins and Flavonoids**

Characterized chemical groups and identified phenolic compounds in Deglet-Nour extracts are indicated in Tables 3-4. The syrup is the richest in condensed tannins and reducing sugars followed by the Aq E; while PE E and Ch E are the richest in sterols and triterpenes. Flavonoids are present in small quantities in extracts which resulted in their discreet highlighting in addition to their low contents estimated by spectrophotometry.

Characterized sterols may correspond to phytoestrogens presumed to be in dates. Indeed, some researchers isolated from date palm stems, by hexane extraction, 3-hydroxyphytosterols, 3,6-diketophytosterols and 3-keto-6-hydroxyphytosterols (Fernández et al., 1983).

Extracts can be classified in descending order according to their total phenols contents as following: Aq E>Ch E> syrup>EA E>PE E. In the case of condensed tannins, they can be classified in the same way to: syrup>Aq E>EA E>Ch E> PE E. while descending classification of extracts according to their flavonoids contents is: Aq E> Ch E> EA E> syrup>PE E (Table 4).

Similar results of total phenols content were obtained by Dhaouadi et al. (2011) for the Tunisian Deglet-Nour aqueous syrup (5480 mg kg<sup>-1</sup>). However, obtained results are clearly superior to the result found by Mansouri et al. (2005) which is 67.3 mg kg<sup>-1</sup> for a crude extract of the same variety from another region of Algeria.

Noting, it's well established today that different phenolic contents of date varieties result from the effect of a number of factors, the main ones being climatic and environmental factors as light; topography and soil type; degree of maturation as well as the harvest period. Extraction and quantification methods also play an important role in this difference.

Content of phenolic compounds includes not only condensed tannins and flavonoids highlighted and quantified in the extracts, but also the phenol acids identified by HPLC analysis, as caffeic, chlorogenic, cinnamic, salicylic and gallic acids. Presence of phenolic acids in dates has been confirmed by several researchers. Mansouri et al. (2005) reported the presence of cinnamic, ferulic, sinapic, coumaric acids and

their derivatives such as 5-ocaffeoylshikimic acid; they mentioned that this high content of free cinnamic acids is not common in other fruits.

Identified flavonoids in studied extracts were rutin, luteolin, quercetin and catechin. Extraction with acetone-water-acetic acid mixture has led to the identification of glycoside flavonoids of California Deglet-Nour such as quercetin, luteolin and apigenin (Hong et al., 2006).

The portion of tannins in total phenolic contents of extracts is considerable and major identified ones were tannic acid and procyanidin B<sub>2</sub> and B<sub>3</sub>. It was reported that ripe dates contain significant levels of proanthocyanidins (Hong et al., 2006); which is in agreement with this study results. Noting, in this context that condensed tannins are difficult to extract, especially if they are highly polymerized; so it is quite possible that their content in Deglet-Nour exceeds what we found as values.

**Table 3:** Characterized Chemical Groups, HPLC Identified Phenolics in Deglet-Nour Extracts

		Extracts				
Chemical groups		PE E	Ch E	EA E	Aq E	Syrup
	Condensed tannins	-	+	+++	+++	++++
	Flavonoids	-	+	+	+	±
	Reducing sugar	-	-	++	+++	++++
	Sterols and triterpenes	+++	+++	+	-	-
HPLC Identified phenolics	Tannic acid	-	+	+	+	++
	Gallic acid	+	-	-	+	+
	Salicylic acid					
	Caffeic acid		-	+	+	-
	Cinnamic acids					
	Chlorogenic Acid	-	-	+	+	-

Rutin	-	-	+	+	+
Luteolin	-	+	+	+	-
quercetin	+	-	+	-	-
Catechin	-	+	+	+	-
Procyanidine B <sub>2</sub>	-	-	-	+	+
Procyanidine B <sub>3</sub>	-	-	+	+	-

(-) no reaction for chemical groups, (-) not identified for HPLC analysis

The very low polyphenol content of non-polar extract (PE E) is undoubtedly due to the fact that these compounds are in glycosidic form within the date which is a very rich fruit in carbohydrates.

**Table 4:** Phenolics Contents (mg kg<sup>-1</sup>) of Deglet-Nour Extracts

Extracts	Total phenols	Condensed tannins	Flavonoids
PE E	836.5± 11.85 <sup>a</sup>	6.49±0.30 <sup>a</sup>	1.19±0.08 <sup>a</sup>
Ch E	5112.1±23.92 <sup>b</sup>	118.1± 6.2 <sup>b</sup>	69.9±1.25 <sup>b</sup>
EA E	2382±7.35 <sup>c</sup>	493.6±4.8 <sup>c</sup>	12.69±0.95 <sup>c</sup>
Aq E	5725±11.48 <sup>d</sup>	543.9±12.8 <sup>d</sup>	200.4±1.00 <sup>d</sup>
Syrup	2845.3±7.48 <sup>e</sup>	909.4±17.7 <sup>e</sup>	7.96±0.03 <sup>e</sup>

Values were expressed as means ± SD (n = 3).

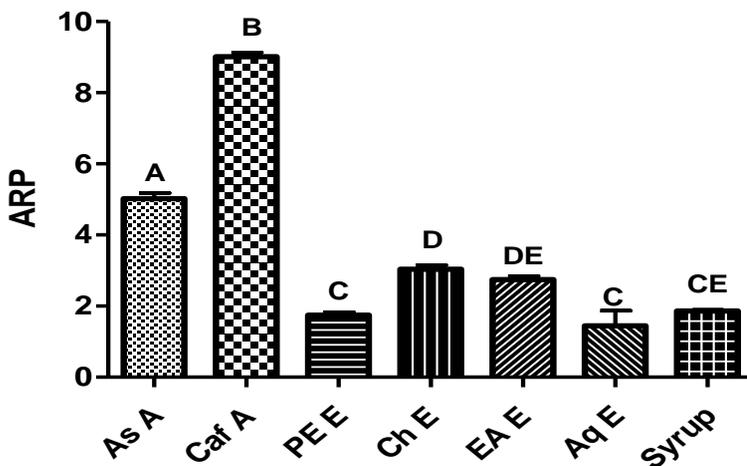
a,b,c: means followed by different letters in the same line are significantly different (p<0.01)

### 3.3. Antioxidant Activity

Presence of antiradical molecules in Deglet-Nour extracts was confirmed, at first, using TLC plates. Results of assessment of this activity amounts against DPPH are showed in Figure 1. Expressed as ARP, all extracts have given antiradical effects; however they were lower than standards ones. This is certainly due to the purity of the standards tested towards crude extracts. Activity of fractioned extracts (EP E, Ch E and EA E) involves mainly their phenolic compounds as correlation between their contents and rates of antiradical capacity is

high ( $r=0.86$ ). Many studies have confirmed the important anti-free radical effect of gallic, caffeic and chlorogenic acids as well as quercetin, rutin, luteolin and catechin, all present in prepared extracts. However, part of this activity can be attributed to carotenoids believed to be extracted by the organic solvents used for fractionation. In fact,  $\beta$ -carotene and its isomers in addition to lutein are the most abundant in mature Deglet-Nour date cultivated in the same area.

On the other hand and despite that the aqueous and syrup extracts are rich in phenols; their anti-free radical activities are lower but still remain considerable. This can be explained by the fact that Folin-Ciocalteu reagent was reduced by sugars abundant in these extracts which further increased the absorbance during the determination of TPP contents which are, in fact, in lower quantity in these extracts. Noting that, Deglet-Nour is rich in reducing sugars at Tamar stage (glucose and fructose). Tannins constitute the largest part of the polyphenols in aqueous and syrup extracts; they are therefore at origin of the anti-free radical capacity of these extracts. Indeed, tannins have a greater antiradical power than that of simple phenols because a single molecule can trap a number of radicals at a time.



**Figure 1:** Anti-radical Power (ARP) of Date Extracts and Standards (Ascorbic and Caffeic Acids (As A, Caf A)). Values were expressed as means  $\pm$  SD (n = 3). Bars with different letters indicate significantly different activities ( $p \leq 0.01$ )

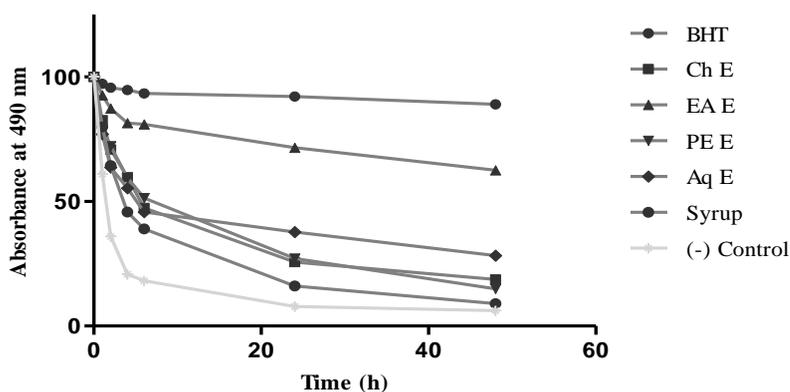
The  $\beta$ -carotene/linoleic acid bleaching technique makes assessed the antioxidant activity of the five extracts by inhibiting lipid peroxidation following absorbance over time.

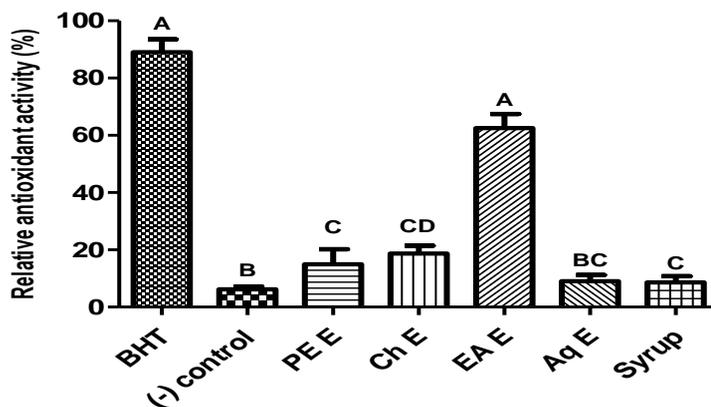
The bleaching kinetics of  $\beta$ -carotene in the absence and in the presence of date extracts and BHT as well as the relative antioxidant activities (RAA) are shown in Figure 2.

In ascending order, organic extracts (PE E, Ch E and EA E) showed significant inhibitory activities. Indeed, the extract E AE gave a level of inhibitory activity of lipid peroxidation comparable to that of BHT standard, as pure antioxidant, without significant difference ( $p \leq 0.01$ ). On the other hand, the syrup gave low activity, while that of Aq E is

lower and comparable to the negative control's one ( $p \leq 0.01$ ) despite their high contents of polyphenols. The correlation test between extracts RAA and their total polyphenols content gave a value of  $r = 0.36$ , in the case of flavonoids and tannins,  $r = 0.1$  and  $r = 0.04$  respectively. Indeed, high contents of polyphenols, flavonoids or condensed tannins do not necessarily reflect a strong antioxidant activity.

Nothing that, the medium for carrying out the  $\beta$ -carotene bleaching test is similar to an emulsion system in water where the nonpolar extracts are concentrated in the lipid-water interface thus allowing the prevention of peroxidation while the water-soluble antioxidants found in polar extracts remain diluted in the aqueous phase and are less active.





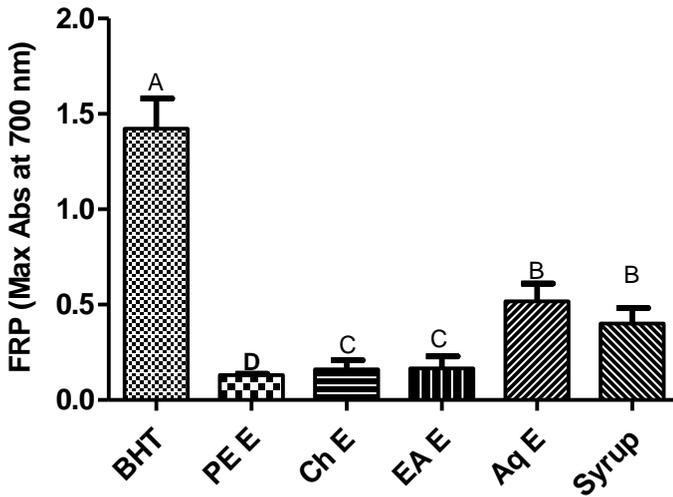
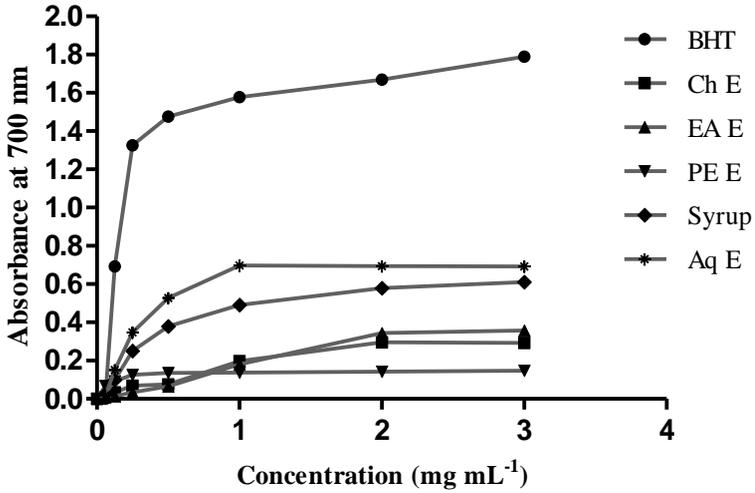
**Figure 2:** Evolution of Discoloration of  $\beta$ -carotene and Relative Antioxidant Activity of Date Extracts and Controls after 48 Hours. Values were expressed as means  $\pm$  SD (n = 3). Bars with different letters indicate significantly different activities ( $p \leq 0.01$ )

The capacity of chloroformic and ethyl acetate extracts to inhibit the oxidation of linoleic acid, suggests that the fat-soluble compounds responsible for this effect are the less polar fraction of the highly methylated flavonoids, and the carotenoids which Deglet Nour contains. On the other hand, we cannot exclude the role of medium polar phenolic compounds from EA E.

The presence of reducing agents in Deglet-Nour extracts caused the reduction of  $\text{Fe}^{3+}$  / ferricyanide complex to the ferrous iron form. Therefore,  $\text{Fe}^{2+}$  could be evaluated by measuring and monitoring the increase in absorbance of the blue color in the reaction medium at 700 nm. The increase in absorbance indicates an increase in the reducing capacity of the extracts which was strongly correlated with the increase in their concentrations and reached a plateau from 1 mg / ml (Figure 3).

Classification of extracts in ascending order, following their relative reducing power is: PE E, Ch E, AE E, Syrup and Aq E with respective values: 8.21, 16.26, 20.11, 34.47 and 38.60%.

The obtained results showed a considerable iron reducing activity of these crude extracts against that of BHT standard which was stronger because of its purity. Extracts reducing power is probably due to the presence of hydroxyl groups of phenolic compounds which can serve as electron donors. In fact, the reducing power of the five extracts was found positively, not strongly, correlated with the polyphenols content ( $r = 0.57$ ). Indeed, it is obvious that this antioxidant power is also due to reducing sugars strongly present in the polar extracts (Table 3). Several studies have shown that the hydroxyl groups of phenolic compounds are responsible for their antioxidant power; also reducing sugar are electron donors in an oxidation-reduction reaction.



**Figure 3:** Evolution of Absorbance and Ferric Reducing Power (FRP) Of Deglet-Nour Extracts and Standard (BHT). Values were expressed as means  $\pm$  SD (n = 3). Bars with different letters indicate significantly different activities ( $p \leq 0.01$ )

### 3.4. Antibacterial Activity

Results of assessment of antibacterial activity of Deglet-Nour extracts are reported in Figure 4. *Staphylococcus aureus* ATCC 25923 was found to be sensitive to all extracts, which resulted in inhibition zones with diameters ranging from 12 mm given by Aq E to 16.5 mm obtained with EA E. While *Staphylococcus aureus* (MRSA) was less sensitive to all extracts in addition to its resistance to Ch E. Its sensitivity was less important than that of *Staphylococcus aureus* and consisted in inhibition zones with diameters varying between 10.5 and 14 mm.

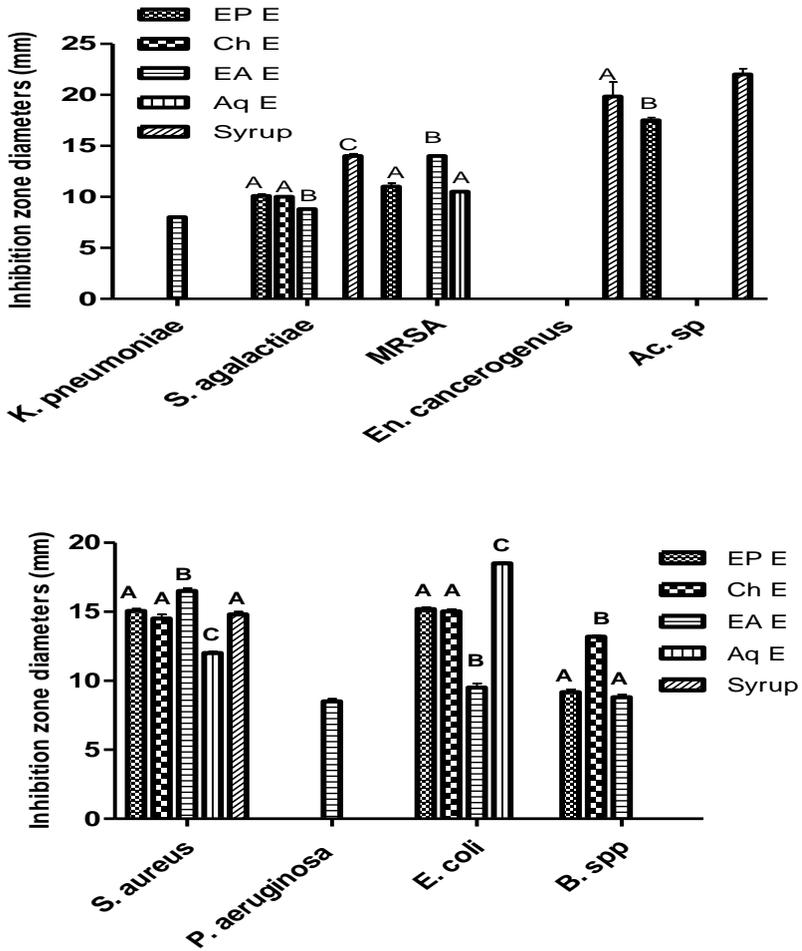
*Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most resistant strains to fruit extracts, in fact they were found very insensitive, only by testing EA E, which gave 8.5 and 8 mm as diameters of inhibition zones of these two strains respectively.

*Escherichia coli* had a large area of sensitivity, 18.5 mm towards Aq E; noting that only the syrup did not inhibit this strain.

On the other hand, *Streptococcus agalactiae* was resistant to Aq E, a little bit sensitive to EP E, Ch E and EA E and fairly sensitive to syrup. Whereas *Bacillus spp* was resistant to Aq E and syrup, a little sensitive to EP E and EA E and fairly sensitive to Ch E.

Only the syrup significantly inhibited *Enterobacter cancerogenus* (19.83 mm). The growth of *Acenitobacter sp* was also considerably inhibited by syrup (22 mm) and EP E (17.5 mm).

From obtained results, it appears that the highest inhibition was exerted by the syrup on *Enterobacter cancerogenus* and *Acenitobacter* sp. This extract is the richest in tannins capable of combining with proteins involved in metabolism and bacterial growth and thus inhibiting their actions. The considerable inhibitory effect of PE E and Ch E with respect *S. aureus* and *E. coli* may be due to the mono and sesquiterpenes besides alcohols, esters and aldehydes constituting date aromas and which are easily extracted by these solvents. In fact, at Tamar stage, Deglet Nour contains appreciable quantities of volatile constituents in its essential oils; the latter have proven antimicrobial properties. Among components of Deglet-Nour aroma were identified by El Arem et al. (2011): important amounts of nonanal, decanal and tridecanal, with two unsaturated derivatives, (Z)-2-octenal and (E)-2-nonenal, detected only in this variety at Tamar stage. It was also found to be rich in saturated hydrocarbons (n-undecane, n-tetradecane and n-hexadecane) and ketones, specifically (E)-geranylacetone, 6-methyl-5-hepten-2-one and 2-undecanone, which give the fruit its characteristic and intense aromas. The sensitivity of the different strains to extracts (Ch E, Ac E and Aq E) is mainly due to their phenolic components; these compounds are potent inhibitors of bacterial growth primarily by combining the vital proteins of microorganisms. In fact, quercetin, rutin, luteolin and catechin have shown, *in vitro* in various studies, a considerable antibacterial effect against several strains including *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumoniae* and *Bacillus spp* (Farhadi et al., 2019).



**Figure 4:** Inhibition zone diameters (mm) of the growth of *tested strains* by Deglet-Nour extracts. Values were expressed as means  $\pm$  SD (n = 3). Bars with different letters for the same strain indicate significantly different activities ( $p < 0.001$ )

On the other hand gallic acid has been found to be a strong inhibitor of *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Borges et al., 2013). Caffeic acid potentiated the antibiotic activity against all bacterial strains when used in research study of Lima et al.

(2016). Chlorogenic acid showed also a strong effect on *Klebsiella pneumoniae*, *Escherichia coli* and *Staphylococcus aureus* in some studies (Naveed et al., 2018).

### **3.5. Haemostatic Activity**

The blood plasma assayed with aqueous extract was recalcified in times lower than that of the correspondent control (Table 5). Diminution percentages of blood plasma time coagulation increased with the increasing of extracts concentrations. This result can be explained by the presence of considerable concentration of condensed tannins in aqueous extracts. These molecules were used, in fact, as adjuvant in coagulation of water purification process (Özacar & Şengil, 2002) and in treatment of landfill (Ibrahim & Yaser, 2019).

However, in spite of high concentration of tannins in syrup, no coagulation was obtained when using these extracts to test the haemostatic activity in the same concentrations (Table 5).

This effect was very surprising because it was permanent. In reality biological activity of tannins is due to their affinity for proteins (Asquith & Butler, 1985; Hagerman & Butler, 1989), but this affinity is high for some proteins and low for others (Hagerman, 1988; Bueno et al., 2008). Otherwise, tannins with an anticoagulant effect were isolated after their combination with thrombin (Dong et al., 1998). As thrombin is directly involved in blood clotting, this can explain probably the anticoagulant effect of Deglet-Nour syrup.

**Table 5:** Results of Plasma Recalcification Test Using Deglet-Nour Macerate and syrup

Samples	Extract (mg)	Coagulation time ( min)	Diminution of plasma time recalcification (%)
Plasma + Aq E	5	5.45±0.16a	1.28±0.75a
	10	5.28±0.12b	4.35±0.10b
	20	5.06±0.06c	8.32±1.00c
Plasma + syrup	5	absence of plasma coagulation	absence of plasma coagulation
	10		
	20		
Plasma control		5.52±0.12	0

Values were expressed as means ± SD (n = 3)

a,b,c: means followed by different letters in the same column are significantly different (p<0.01)

## CONCLUSION

Deglet-Nour used in the study is of good food quality regarding its physicochemical characteristics. Its extracts, containing appreciable amounts of phenolic compounds, gave high levels of antibacterial and antioxidant activity. Moreover, the syrup exhibited a considerable lasting anticoagulant effect which can be used for dietary supplementation of elderly instead of aspirin. The involvement of fruit phenolic compounds in these activities was considerable but not very important, which made it possible to envisage the participation of other molecules in the appearance of the studied biological effects.

## ACKNOWLEDGMENTS

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## CHAPTER VIII

### DROUGHT STRESS IN PLANTS AND ITS RELATIONSHIP WITH PLANT HORMONES

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## **INTRODUCTION**

In today's world, there are some situations caused by increasing climatic changes and the stress factors that plants are exposed to is one of them. With these changes increasing day by day, it is thought that there will be great difficulties in plant growth and crop yield in many countries. Drought stress caused by climatic changes plays a restrictive role especially in plant development. Many studies are still being conducted on the relationship between the physical, metabolic and biochemical activities of plants against biotic and abiotic stress factors and their hormonal effects. Hormones are organic compounds that are naturally synthesized in the plant, can be transported to other parts of the plant from the place where they are synthesized, have special effects, can show their effects even in very low concentrations, and regulate growth and development (physiological events). Therefore, the level of hormones in plants exposed to stress gives us an idea about how the plant's development and defense mechanisms can work. In this review, the effects of drought stress, which causes significant losses in plant growth and development and productivity, and hormones, which are natural defense compounds in the plant, are explained.

Plants are constantly faced with environmental constraints of both biotic and abiotic origin. Abiotic stresses that terrestrial plants are most exposed to are common environmental stress factors such as drought, salinity and extreme temperatures (Seki et al., 2003). There are many stress factors caused by complex environmental conditions and abiotic stress factors occur as a result of them. These are strong light,

ultraviolet, high and low temperature, freezing, drought, salinity, heavy metals and insufficient oxygen status (Hirayama & Shinozaki, 2010). Stress factors caused by increasing climatic changes continue to occur. The increase in these changes negatively affects plant growth and crop yield in many countries (Denby & Gehring, 2005). In agricultural terms, drought is related to the amount of water that the plant planted in the field can get from its roots during the growth period, rather than the total amount of precipitation throughout the year. Significant losses occur in terms of development and especially yield in plants that experience water deficiency during the growth period (Tuberosa, 2012; Turner et al., 2014).

Plant defense against all kinds of abiotic (low and high temperatures, lack or excess of nutrients, air pollution, heavy metals, drought, salinity and radiation) and biotic (virus, bacteria, disease-causing fungi, etc.) stress factors in nature developing mechanisms. Plants try to continue their growth and development by adapting to these stress conditions. In stressed plants, reactions occur within the framework of genotypic characteristics. Some plant species and varieties are slightly affected by stress, and some are fatally damaged by stress. It is known that this type of different adaptation abilities based on genetic foundations, as well as factors such as different growth periods, severity and genus of plants, are effective on defense mechanisms in plants (Yaşar, 2003; Yaşar & Ellialtıođlu, 2008; Yasar et al., 2008; Yasar et al., 2010).

Plants can be exposed to climatic changes such as drought, salinity, excessive precipitation, heat or cold during their life processes. Abiotic stress conditions resulting from these changes directly affect the growth and development of plants (Taiz & Zeiger, 2010). Abiotic stresses such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress reduce agricultural activities and cause serious threats to the ecological balance. However, abiotic stress has been the main cause of crop yield loss worldwide and threatens the future of the agricultural industry by reducing the average production of important agricultural products by approximately 50% (Wang et al., 2004; Mahajan & Tuteja, 2005). Drought is one of the abiotic stress conditions that significantly affect the growth and development of plants. Many physiological characteristics that determine yield in plants are also affected by drought conditions. (Farooq et al., 2009).

Plant growth is significantly affected in drought conditions. This effect on plant growth is dependent on the duration of the water shortage. In the first periods of dry conditions, the plant slows down stem elongation and triggers root development in order to reach more water. On the other hand, both stem and root development cease if dry conditions last long enough to cause damage to the plant. In addition, plant leaf area and number of leaves decrease. Even some leaves of the plant turn yellow and fall off. The decrease in plant growth continues due to the cessation of cell division and expansion of cells in shoot and root meristems. The cessation of cell division or expansion is directly related

to the decrease in the rate of photosynthesis due to water deficiency (Anjum et al., 2011).

Drought stress activates many protection systems at physiological, biochemical and molecular levels in plants. Drought conditions inhibit plant growth as a result of reducing cell division and growth. In addition, the decrease in turgor pressure, which is a driving force for growth, and the negative effect of transpiration during drought may also cause a decrease in mineral substance uptake and a decrease in growth rate. For this reason, drought stress is the most important limiting factor for agricultural production, but with the effect of global warming, this situation leads to more serious negative consequences (Capell et al., 2004).

Plants exposed to drought stress create many physiological, biochemical and molecular responses and accordingly, they can develop tolerance mechanisms that will enable them to adapt to limited environmental conditions (Arora et al., 2002). It is reported that some internal and external factors as well as environmental conditions play a role in the normal development of the plant and its ability to respond to these adverse conditions (Kumlay & Eryiğit, 2011).

Plants need chemical communication between cells to survive under stress and grow under these conditions over time. As a result, they develop different mechanisms. One of these mechanisms is hormonal interactions. These hormones are chemical messengers produced in one part of the plant and transported to other parts. Here, they play critical

roles in regulating plant responses to stress at extremely low concentrations (Öktüren & Sönmez, 2005).

Plant growth regulators come in two forms, natural and synthetic. While natural hormones are synthesized by the plant, synthetic hormones are substances with different structures developed by the chemical industry. Synthetic hormones have similar effects with natural hormones, and in some cases they may have more effects (Çetin, 2002). Hormones provide the development of the defense systems of plants against adverse conditions and play an important role in the perception of changes in environmental factors (Davies, 1995). Hormones support the response of plants to abiotic stress factors such as photooxidative stress, high temperature stress, water scarcity, drought, soil salinity, cold and frost stress, injury, mechanical stress and air pollution (Vickers et al., 2009). Hormones that promote plant growth and development are called stimulators. This group includes auxin, cytokinin and gibberellin hormones. There are hormones in this group that have opposite effects, retarding growth and development, and slowing them down, and these are called inhibitors. Abscisic acid (ABA) and ethylene hormone are included in this group. Most of the physiological activities that take place in the plant occur with the control of hormones in the stimulator and inhibitor groups (Bozcuk & Topçuoğlu, 1982).

Until recently, plant growth was thought to be regulated by only five types of hormones: auxins, gibberellins, cytokinins, ethylene, and abscisic acid. However, there are currently data showing the existence of brassinosteroids, which are plant steroid hormones. Brassinosteroids

have a wide range of morphological effects on plant growth. A wide variety of other signaling molecules have also been identified, including jasmonic acid, salicylic acid, and the polypeptide system, which play a role in resistance to pathogens and defense against plant pests. Therefore, the number of hormones and hormone-like signaling agents in many numbers and types in plants continues to increase (Taiz & Zeiger, 2008).

## **1. AUXIN**

Auxins were the first to be discovered among plant growth regulators. The discovery of the hormone auxin dates back to Charles Darwin in 1880. Later, various experiments on hormones were conducted by Boysen-Jensen in 1913 and by Paal in 1919 (Leopold, 1963; Santner et al., 2009). However, in 1928, Went first determined this substance that is effective in growth and named it auxin (auxine), similar to auxeme in the Greek meaning of growth. Auxins are synthesized in plants mainly in meristematic tissues in root and stem tips, cambial tissues, fruit, flowers and growing leaves. However, auxins are also found in other parts of the plant apart from these plant parts (Kadıoğlu, 2004). Auxins are divided into two groups, natural and synthetic. Naturally synthesized auxins consist of Indole-3-acetic acid (IAA), indole-3-ethanol, indole-3-acetaldehyde, and indole-3-acetonitrile. In addition to these, indole butyric acid (IBA) and naphthalene acetic acid (NAA) are known as synthetic auxins (Özen & Onay, 1999). Auxins are examined under 4 groups and the important growth regulators are as follows.

**a) Indole group**

Indole acetic acid (IAA)

Indole proionic acid- (IPA)

Indole butyric acid (IBA)

**b) Naphthelen group**

Naphthelen Acetic Acid (NAA)

P - Naphthoxy acetic acid (NOA)

**c) Phenoxy group**

Phenoxy acetic acid (FOAA)

Phenylacetic acid (FAA)

4 -Chlorophenoxyacetic acid

2,4-Dichlorophenoxy acid (2,4-D)

2,4,-5-Trichlorophenoxy acetic acid (2,4,5-T)

**d) Benzole group**

2.4.6- Trichlorobe benzoic acid

2.3.6- Trichlorobenzoic acid

4- Amino-3,5,6-Trichloropicolinic acid

The effects of these substances on plants are as follows: To encourage root formation of cuttings, to provide parthenocarpic fruit set, to provide adventitious root formation, to prevent fruit and leaf fall, to increase fruit set, to enable buds to bloom earlier, and to prevent weed growth (Çetin, 2002).

Depending on age and species, auxins generally increase adventitious and primary root formation at low concentrations, promote female flower formation, seed germination, parthenocarpic fruit formation, and assimilate transport in the phloem. They also regulate the development of ovaries in flowers by increasing ethylene synthesis (Salisbury & Ross, 1991). It has been suggested that auxins may also play a role in resistance to diseases by affecting the phenol metabolism of plants (Skoog & Montaldi 1961).

Davis & Diamond (1953) suggested in their studies that auxins may cause reductions in diseases in plants by increasing the soluble sugar content in plants. Vidhyasekaran (1975) reported that only indole acetic acid (IAA) among auxins was detected in millet (*Eleusine coracana* L.) leaves and young millet leaves containing more IAA were resistant to fungal pathogens *Helminthosporium nodulosum* and *H. tetramera*. White clover plants under arid conditions IAA application has been made and it has been determined that IAA has an important role in preventing drought damage in these plants (Zhang et al., 2020).

## **2. CYTOKININ (CK)**

Cytokinins were discovered as a result of studies to find factors that stimulate division activities in plant cells. Cytokinins have been found to control many other physiological and developmental events as well as cell division in plants. These effects include delaying senescence in severed organs, enlargement of cotyledons, transport of nutrients, chloroplast maturation, control of morphogenesis. In summary, cytokinins have different regulatory roles in higher plants that are not related to each other (Kadioğlu, 2004). Cytokinins (CK) are mainly found in plant roots and shoots. CK is responsible for cell division here known as a group of phytohormones.

Excessive cytokinin accumulation occurs in transgenic tobacco lines exposed to severe drought stress. Cytokinin hormone, PSI, PSII and cytochrome b6f (Cytb6f) increases the expression of photosynthetic genes encoding the complex. observed (Rivero et al., 2010).

Cytokinins are organic substances in the structure of kinin, which emerge especially during cell divisions and, unlike other hormones, are included in the structure of both plants and animals. Cytokinins synthesized naturally in plants consist of zeatin, dihydrozeatin, isopentenyl adenine, dimethylalladenine, and synthetic cytokinins consist of kinetin (N<sup>6</sup>-furfurylamino purine), benzyladenine (BA) and tetrahydropyranlybenzyl adenine (PBA) (Ünsal, 1993). There are more than 200 natural and synthetic cytokinins chemically and biologically (Salisbury & Ross, 1991).

Zeatin, one of the natural cytokines, was first obtained from corn grains in studies. Then, with the addition of coconut milk to tissue culture media, an increase in cell divisions occurred and it was observed that coconut milk contained zeatin (Kadioğlu, 2004).

The most common cytokinin that occurs naturally in higher plants is zeatin. In addition to stimulating cell division, cytokinins also act on shoot and root differentiation, lateral bud growth, leaf development, chloroplast development, and senescence in tissue culture. All of these events usually occur when used with auxin. For this reason, mixtures of auxin and cytokinin are added to the nutrient media (Özen & Onay, 1999).

### **3. GIBBERELLINS (GAs)**

Gibberellins (GAs) are hormones that generally have effects on growth and development in plants. They control seed germination, leaf expansion, stem elongation and flowering in the plant (Magome et al.,

2004). While the GA hormone controls plant growth and development, it has long been known that there is an interaction between other signaling hormones or environmental stresses. Recently, GA-assisted stabilization of DELLA proteins is modulated by environmental signals (such as salt and light) and other plant hormone signals (such as auxin and ethylene), revealing the mechanisms of this cross-interaction at the molecular level (Achard et al., 2006). The biosynthesis of GAs is regulated by both developmental and environmental stimuli (Yamaguchi & Kamiya, 2000; Olszewski et al., 2002). Gibberellic acid (GA) accumulates quickly when plants are exposed to both biotic (McConn et al., 1997) and abiotic stresses (Lehmann et al., 1995). For example, gibberellic acid (GA<sub>3</sub>) has been reported to help increase growth in wheat and rice plants under salt stress (Parasher & Varma, 1988; Prakash & Prathapasenan, 1990).

Maggio et al. (2010) reported that the application of GA<sub>3</sub> to the tomato plant reduces the stomatal resistance of the plant and increases the use of low salinity plant water.

Gibberellins are the most important hormones affecting the growth in plants (Kadioğlu, 2004). It was first discovered in 1926 by Japanese scientists in the fungus called *Gibberella fujikuroi*, which causes too much grading in the rice plant and got its name from there. Later, a sterile extract was obtained from this mushroom and named as gibberellic acid (GA<sub>3</sub>) (Vardar, 1970; Kılıç, 2007; Morsünbül, 2010). The most widely used gibberellins are GA<sub>3</sub>. Today, it is known that there are more than 126 types of gibberellin. Gibberellins are found in

large amounts in buds, embryos, roots, young leaves, flowers, fruits, and various parts of the plant such as the cambium. Among the gibberellins, GA has a great commercial importance (Baktır, 2010).

Gibberellins have important effects on different physiological developments related to increasing yield and quality. Among these, they are very effective in breaking seed and bud dormancy, parthenocarpic fruit formation, increasing fruit set and fruit development, eliminating genetic stunting, eliminating the need for chilling, synthesizing some enzymes (amylase) and promoting germination (Kaynak & İmamgiller, 1997; Olszewski et al., 2002). The most obvious effect of gibberellins in plants is to increase the elongation of cells (Tyler et al., 2004).

Due to the growth-promoting effect of gibberallic acid, it is widely used in table varieties, especially in grape plantings. According to Zweig (1967), the use of GA<sub>3</sub> in vines causes the grape grains to become larger, and besides this, the lengthening of the cluster and the increase in the sparseness between the grains after the applications made for cluster thinning reduce the fungal developments that may occur.

#### **4. ABSCISIC ACID (ABA)**

There are also natural substances that prevent growth and development, which act in the opposite direction of natural substances that promote growth in plants. Abscisic acid (ABA) is the most important of these substances (Morsünbül et al., 2010). ABA has a reducing effect on the growth and functioning of metabolism in plants under all stress

conditions, and as a result, it ensures the protection of the resources available in the plant, thus reducing the rate when the stress is removed and the metabolism becomes normal (Jacksons, 1985).

ABA synthesis increases in plant tissues exposed to stress conditions and ABA synthesized in chloroplasts is rapidly transported to other parts of the plant (Özen & Onay, 1999). Abscisic acid provides tolerance to plants under different abiotic stresses such as drought, salinity and low temperature stresses (Giraudat et al., 1994; Chandrasekar et al., 2000; Reddy et al., 2004), stomatal opening (Hartung et al., 1998). indicated by the studies it promoted. The amount of ABA increases during stress in plants under water stress, causing the closure of stomata and slowing down protein synthesis. In addition, ABA acts as an inhibitory substance in the dormancy of storage organs such as seeds, buds and tubers, and ensures the storage of protein synthesis in seeds (Seçer, 1989; Çetin, 2002).

ABA is a natural antagonist of auxins, gibberellins and cytokinins, which are growth and development-promoting hormones (Kumlay & Eryiğit, 2011). However, it is known that abscisic acid (ABA) acts as a mediator in plant responses to many stress factors, including drought and salt stress. ABA is also an important signaling molecule that enables plants to survive in adverse environmental conditions such as salt stress (Keskin et al., 2010). Since the salinity stress caused by the increase in the salt content in the soil also affects the water uptake by the roots and leads to a decrease in the water potential, especially the drought and salinity stress signal transmission mechanisms are almost

the same. It is known that the water shortage perceived by the roots is transmitted to the stem by factors such as abscisic acid (ABA), cytokinins, ethylene and malate (Anjum et al., 2011). It is also known that abscisic acid accumulation causes gene expression changes and plays an important role in the restructuring of metabolism during drought stress (Shanker et al., 2014). Transient increases in ABA levels lead to numerous changes, such as induction of gene expression, accumulation of compatible soluble and protective proteins, increased levels of antioxidants, and suppression of energy consumption pathways (Bartels & Sunkar, 2005; Taiz & Zeiger, 2010).

It has been reported that the stress responses of root and shoot tissues are coordinated with the increased amount of hormone in the xylem sap acting by "root to shoot" communication (Davies et al., 1994). However, some speculation remains regarding the ability of ABA to act as a signal mediating stress effects occurring in the root zone (Jia et al., 2002). There is substantial evidence that ABA attracts from the root to the xylem sap and acts as a stress signal. One study showed that ABA contributes to an increase in xylem water potential and plant water uptake in the presence of salt (Fricke et al., 2004). It has also been reported that the increase in ABA concentration in the xylem is associated with general inhibition of leaf growth and decreased leaf conductivity (Jeschke & Hilpert, 1997).

As a result of the studies on abscission, a substance that stimulates abscission from cotton fruits was purified and crystallized and named abscisin II ( $C_{15}H_{20}O_4$ ). Because 2 years ago, the substance isolated from

mature cotton bolls, which was determined to have an accelerating effect on abscission, was named abscisin I. At the same time, a substance that induces bud dormancy from prickly pear leaves was purified and named dormin. Later, dormin was chemically defined and abscissa was found to be similar to H. In 1965, it was determined that dormin and abscisic II were the same substance. In 1967, the Addicot, Wareing and Comforth research groups working on this issue came together and decided that it would be appropriate to use abscisic acid for its name and ABA for short. The name abscisic acid originated from the abscission event and for years it was thought that ABA was primarily responsible for abscission stimulation (Kadioğlu, 2004).

Research has been done on the role of ABA in water, salt and freezing stresses, and it has been seen that ABA acts as a stress hormone. It was noted that under drought conditions, ABA concentrations in leaf parts were 40 times higher, and that such a change could not occur in any hormone concentration in response to an environmental cue. ABA encourages stomatal closure, thereby reducing water loss by transpiration (Kadioğlu, 2004).

With the increase of ABA in the leaves, the stomata are closed and the transpiration and shoot development decrease. It has also been observed that low concentrations of abscisic acid increase the water taken from the roots and thus reduce the water stress in the shoots (Loveys & Kriedemann, 1974). Under water-deficient conditions, abscisic acid (ABA) plays an important role in the response and tolerance to dehydration (Reddy et al., 2004).

## 5. ETHYLENE

Unlike other hormones, ethylene ( $C_2H_4$ ) is an organic molecule with a simple structure and exists as a gas at room temperature. It is known as the maturation hormone in plants and causes physiological effects in the plant even if it is present in very low amounts. Ethylene is an important hormone that is effective in large amounts in taste, color, texture and structure of horticultural plants. Ethylene is synthesized from all organs according to the development status of the plant and is synthesized from mature and aging tissues that are mostly under stress. It is reported that ethylene is synthesized in the highest amounts in leaves and flowers in the periods before wilting and shedding. At least ethylene is synthesized by the roots of the plant (Çetin, 2002; Öktüren & Sönmez 2005; Baktır, 2010).

In addition, the events caused by ethylene in plants are as follows; Breaking dormancy, leaf and fruit shedding, promoting flowering in some plants, stimulating adventitious root formation, promoting female flower formation in monoic plants and facilitating mechanical harvesting by promoting abscission (Raven et al., 1992; Davies, 1995). It has also been observed that ethylene production increases with the effect of stress factors such as air pollution, root pruning, plant pathogens, transplantation and drought in plants (Jacksons, 1985).

It has been observed that ethylene synthesis in plants is increased in some cases. These have been noted to increase in conditions such as fruit ripening, flower senescence, IAA, injury, frost damage, ozone exposure, mechanical injury, drought, and graft water stress. Ethylene

biosynthesis is stimulated by various factors, including developmental stage, environmental conditions, other plant hormones, and physical and chemical harmful substances. It has been observed that ethylene production within the plant increases in some biotic stress conditions. Infection and disease occur when there is a genetically compatible relationship between the host and the pathogen. However, ethylene production generally increases in response to pathogen attack in both compatible (disease-causing) and incompatible (non-disease-producing) relationships. Obtaining ethylene-insensitive mutants has led to the elucidation of the role of ethylene in the response to various pathogens. It has been understood that the role of ethylene in pathogen transmission is complex and dependent on a specific host-pathogen relationship. For example, inhibition of the response to ethylene did not affect the resistance of *Arabidopsis* to *Pseudomonas* bacteria or the tobacco plant to tobacco mosaic virus. However, when the relationships of these pathogens and hosts are compatible, elimination of the ethylene response inhibits the development of disease symptoms even if it does not affect the growth of the pathogen. On the other hand, ethylene, together with jasmonic acid, is required for the interaction of some plant defense genes. In addition, mutants of ethylene-insensitive tobacco and *Arabidopsis* are susceptible to some necrotrophic (cell-killing) fungal pathogens in soil that are not normally plant pathogens. Thus, ethylene appears to be involved in the development of resistance to some, but not others, pathogens (Taiz & Zeiger, 2008).

## **6. OTHER HORMONES**

In addition to the main 5 hormone groups that affect growth and development, there are various growth regulators. These are salicylic acid, jasmonates, brassinosteroids, and polyamines.

### **6.1. Salicylic Acid (SA)**

It is also known for its plant hormone and pain reliever properties. Salicylic acid gets its name from the willow *Salix*, whose analgesic (pain-killing) properties were known before it was chemically isolated. It was first extracted from the bark of willow (*Salix* sp.). It has been reported that salicylic acid, which is found in the majority of plants, helps to defend against biotic stresses, namely insect damage and pathogens such as viruses. The concentration of salicylic acid increases in the infected plant and spreads within the plant. It is thought that salicylic acid binds to a cell receptor and activates genes encoding infection-fighting proteins and provides wound healing (Öktüren & Sönmez, 2005).

The role of SA in defense mechanisms against biotic and abiotic stresses has been well established (Gautam & Singh 2009; Loutfy et al., 2012). It has been stated that salicylic acid (SA) is effective in the formation of metabolic and physiological responses of plants against most stress factors such as salinity, drought, temperature and heavy metals. Therefore, it has been reported that there is an intrinsic growth hormone that affects plant growth and development in the regulation of these responses (Hayat et al., 2010).

The effects and role of SA in physiological and biochemical events occurring in plant systems have been demonstrated by most studies (Raskin, 1992; Raskin, 1995). Salicylic acid is an important signal molecule that is effective in the response of plants to stress factors (Senaratna et al., 2000).

SA is a signaling molecule that has an important role in the plant defense mechanism against many pathogens (Snyman & Cronjé, 2008). It provides plants with features such as response to biotrophic pathogens, induced defense response, and systemic acquired immunity. Salicylic acid has a great importance in physiological events such as plant growth and development, photosynthesis, stomatal regulation, respiration, flowering, senescence and ion uptake (Vicente & Plasencia, 2011).

As a result of the exogenous application of salicylic acid, auxin and cytokinin levels did not change in salt-stressed wheat plants, cell division progressed in the root apical meristem and increased productivity (Shakirova et al., 2003).

It has been observed that the application of SA to the leaves of the corn plant affects some stress factors. So much so that it eliminated the harmful effects of salt stress on the corn plant. SA applied to the soil has been reported to have a curative effect on the viability of maize plants during salt stress (Khodary, 2004).

## 6.2. Jasmonic Acid (JA=MeJA)

The detection of jasmonates includes esters of jasmonic acid (JA) and methyl jasmonate (MeJA), which were first obtained from the jasmine (*Jasminum grandiflorum*) plant. It is a fragrant compound (Meyer et al., 1984; Fan et al., 1998). In previous studies, it has been determined that JA and its fragrant ester, MeJA (methyl jasmonate), have an inhibitory effect on plant growth. Jasmonic acid has been found to exist in approximately 206 plant species together with ferns, mosses, some fungi and algae (Meyer et al., 1984). It has been determined that MeJA has an important role in signaling molecules in plant genes, and it has been determined that it especially increases the presence of some specific plant genes. Especially in case of damage and injury to the plant, it is effective in inhibiting enzymes that cause the breakdown of proteins (especially vegetative storage proteins in soybean) and in the formation of response genes (Van den Berg & Ewing, 1991; Staswick, 1992). Jasmonates have both inhibitory and stimulating aspects. Externally applied jasmonates; photosynthesis, pollen germination, longitudinal development, root growth, flower bud formation, embryogenesis, germination of non-resting seeds as well as protein synthesis, elimination of resting need, adventitious root formation, It has been determined that it has stimulating properties such as abscission, closure of stomata during stress, germination of seeds at rest, ethylene synthesis and subsequent fruit ripening (Fan et al., 1997; Yılmaz & Yıldız, 2001). It was revealed in a study conducted on strawberry plant that JA reduced the negative effects caused by water stress (Wang, 1999). It has been reported that JA and MeJA activate the

enzymes by stimulating the genes to which the enzymes in the protein structure that protect plants against biotic and abiotic stress factors are bound (Hilda et al., 2003). The places where jasmonates are synthesized in the plant; are plant parts such as flowers, leaves, roots and immature fruits (Baktır, 2010).

Plant growth regulators are known to cause various physiological reactions in the plant. Various studies have been carried out in recent years regarding the role of these properties of growth regulators in plant pathogen interaction, increasing the resistance to diseases by stimulating the plant defense system and their role in pest control. Jasmonic acid signaling works particularly well in defense against herbivores. In many studies, it has been reported that some defense properties are gained by JA against biotic stress factors that plants are exposed to. These are the response to necrotrophic pathogens, the induction of anti-herbivorous responses, the production of volatile compounds induced by herbivores, and the stimulation of other organs against the attack, thereby making the plant parts, especially the leaves, inedible and preparing the plant for attack. JA and SA are effective in local and systemic defense against pathogens (Taiz & Zeiger, 2008).

When plants are exposed to stress factors such as drought, excess water, heavy metals, salinity, low and high temperatures, they experience a noticeable change in physiological events occurring within the plant. Hormones have proven in many studies that plants gain resistance against these adverse conditions.

Abiotic and biotic stresses are the main cause of crop loss. Under stress or other adverse climatic and ecological conditions, the hormonal level in the plant can be changed by applying external growth regulators and the plant can become more active in the face of adverse conditions it is exposed to. The effects of auxins, cytokinins, gibberellins, ethylene, abscisic acid, etc., which are widely used in plant production, have been demonstrated by various studies.

### **6.3. Brassinosteroids**

Brassinosteroids (BR) were first described in pure form in 1979 by Grove et al. It was isolated from the pollen of the rapeseed (*Brassica napus*) plant. Brassinosteroids in the steroid structure are named after the Brassica genus of the Cabbage family (Cruciferae). Although the synthesis of BRs is not fully explained today, the starting material is mevalonic acid. BRs are hormones that are very effective in plant stem elongation. For this reason, BR applied externally to some dwarf mutant plants that cannot produce steroids significantly promotes stem elongation in the plant. For example, in a study in which BR was applied to dwarf bean plants, it was reported that height growth was observed as a result of cell division and cell elongation (Clouse, 2009; Baktır, 2010).

Brassinosteroids have been tested for biological activity in many systems for evaluation. It has been determined that it provides resistance to plants against cold, diseases and pests and salt stress, encourages the plant in events such as seed germination, elongation,

root growth, and also increases the product and prevents fruit shedding (Kim, 1991; Nasar, 2004).

Abiotic stress causes morphological, physiological, biochemical and molecular changes in plants. The positive role of BRs in the plant's response to stress has been confirmed by many studies (Divi et al., 2010). Application of 24-epiBL to tomato plants exposed to drought stress decreased H<sub>2</sub>O<sub>2</sub> content and lipid peroxidation, while increasing proline, soluble protein content and antioxidative enzyme activity (Behnamnia et al., 2009).

In some studies, the effects of BRs against combined multiple abiotic stresses were also investigated. Application of 24-epiBL increased drought and cold stress tolerance in *A. thaliana* and *B. napus* seedlings and also helped to overcome seed germination inhibition due to salt stress. Ultimately, the ability of 24-epiBL to increase tolerance to stresses was confirmed by analysis of drought and cold stress marker genes (Kagale et al., 2007). BRs are also used for many purposes such as providing tolerance to salt stress, cold and disease-pests, preventing fruit set, increasing yield, promoting germination and promoting root growth (Rao et al., 2002). The fact that BRs increase plant tolerance to salinity, drought, heat, cold and oxidative stress factors is partially explained by the correlation of expression of stress marker genes such as heat shock protein (hsp) genes, RD29A and ERD10 in BR treated plants. It is thought that BRs also regulate the plant's response to stress through signal exchange with other hormones (Divi et al., 2010).

#### **6.4. Polyamines**

Among the polyamines; diamine, putrescine, triamine, spermidine, and tetramine are ubiquitous in plant cells of sperm (Smith, 1970; Bagni & Pistocchi, 1992). The most important forms of polyamines are; putrescine, spermidine and spermine. In plants, polyamines play a role in cell division, root, adventitious shoot, flower and fruit ripening. They are also effective in growth and development events such as embryo formation in tissue culture. The increase in the levels of polyamines in plants occurs especially in response to water deficiency, salt stress, acid stress, oxygen deficiency, plant aging and environmental stresses (Flores, et al., 1989; Galston & Kaur-Shawhney, 1995; Eti, 2006).

#### **CONCLUSION**

It is very important to protect the existing food production and increase plant production in order to combat the increasing global climate change and the drought experienced as a result of it, in terms of ensuring the continuity of the life quality of human beings. Although the responses of plants to drought stress have been understood to a great extent as a result of long-term research, the positive effects of some hormones should be considered in order to increase the tolerance of plants to drought stress, and it is inevitable that studies on the use of these hormones should be carried out at a more adequate level. This situation reveals the necessity of approaching research with new perspectives and increasing the number of target-result-oriented studies that can be directly transferred into practice. The complexity of plants' responses to drought stress is due to the fact that their responses to other

abiotic stresses, such as temperature and salinity, use similar signal transduction pathways and mechanisms. For this reason, it is important to carry out studies to increase plant stress tolerance, not only to respond to a single abiotic stress, but also to understand the effects of multiple stresses, in order to achieve positive developments. Studies in this area will also shed light on the determination of varieties that will adapt to changing climatic conditions and cope with stress factors. Understanding the hormonal response of plants to stress is essential for increasing agricultural yield.

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## CHAPTER IX

### SALT STRESS IN PLANTS AND ITS EFFECTS ON PLANT HORMONES

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## **INTRODUCTION**

Plants must be able to keep down stress. They should also increase their tolerance level when under stress. Therefore, over time, they develop different mechanisms to grow under these conditions. In this case, the mechanism they develop differs depending on the stress factors. Plants have used a wide variety of mechanisms in which the formation of signaling pathways and the exchange of signaling particles are of major importance. Signaling particles were biochemical products that were taken up by plant receptors, usually in the cell membrane, resulting in the expression of different genes, including stress genes. Such signaling particles help the plant be patient to and survive under stress (Hamel et al., 2006; Colcombet & Hirt, 2008; Miransari & Smith, 2007; Miransari & Smith, 2008; Miransari & Smith, 2009).

Plants were exposed to various adverse conditions that restrict their development in the areas where they live. The conditions that affect or prevent growth, development and metabolism in plants were called stress (Gürel & Avcıoğlu, 2001). Stress factors often show their effects on plants simultaneously and in combination (Kalefetoğlu & Ekmekçi, 2005). Stress factors are divided into two groups according to their origins: These are abiotic and biotic stress factors. Stress factors in plants are tested by scientific studies. Abiotic stress is defined as cold, heat, drought, salinity, excess water, radiation, various chemicals, oxidative stress, wind and nutrient deficiency in the soil. Abiotic stress factors can be further increased. Biotic stress factors are viruses, bacteria, fungi, pathogens, insects (Mahajan & Tuteja, 2005).

There are more than 800 million hectares of land in the world. 7% of these lands are affected by salinity (FAO, 2008). Scientific studies have aimed to regain these lands for agriculture. Some of the soils affected by this salt are due to natural causes (bedrock erosion, ocean salts carried by the wind, etc.). For this reason, a significant part of the recently cultivated agricultural land has become saline. This shows that the cultivated area has decreased significantly. This situation makes people very nervous. It causes the water table to rise and the salt levels to increase in the underground organs of the plant (Munns & Tester 2008).

The salinity of the soil as a result of the increase in the amount of  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Mg}^{+2}$ ,  $\text{Ca}^{+2}$  and  $\text{Cl}^-$ ,  $\text{SO}_4^{-2}$ ,  $\text{HCO}^{-3}$  resulting from more water-soluble salts  $\text{NaSO}_4$ ,  $\text{NaHCO}_3$ ,  $\text{NaCl}$  and less water-soluble salts such as  $\text{CaSO}_4$ ,  $\text{MgSO}_4$  and  $\text{CaCO}_3$  such as  $\text{MgCl}_2$  were one of the main abiotic stresses that reduce plant growth and productivity of many crops worldwide. Salinity can be caused by natural factors (e.g. mineral erosion in the soil), but can also be caused by human activities (irrigation with high mineral water and/or ineffective drainage of the irrigated area, fertilizer application, etc.). Often the soil salinity increases. With the presence of  $\text{NaCl}$  in water and soil, other salts may also play a role. The detrimental effects of  $\text{NaCl}$  were complex and include ion toxicity, hyperosmotic stress, and can cause subsequent stresses such as nutritional imbalances and oxidative stress (Zhu, 2001).

The metabolic toxicity of  $\text{Na}^+$  was mainly due to its ability to compete with  $\text{K}^+$  for target sites with important cell functions. More than 50 enzymes were activated by  $\text{K}^+$ , and high  $\text{Na}^+$  levels or high  $\text{Na}^+/\text{K}^+$  ratio can disrupt a number of enzymatic processes in the cytoplasm. Protein biosynthesis also requires appropriate levels of  $\text{K}^+$  for proper tRNA binding to ribosomes, and high  $\text{Na}^+$  levels can impair this process (Tester & Davenport, 2003; Bartels & Sunkar, 2005). Increasing  $\text{Na}^+$  concentration can cause a hyperosmotic stress by preventing the plant's water uptake, which causes the so-called "physiological drought" (Türkan & Demiral, 2008).

In agriculture, salinity has been an important limiting factor in food production. Soil salinity was known to restrict land use and limit crop yields. Various environmental programs conducted around the world estimate that approximately 20% of these programs were farmland and 50% of the world's fields were salt-stressed (Munn and Tester 2008). Thus, current soil salinity was a major problem for food security. Increased levels of water-soluble salts, e.g.  $\text{NaCl}$ ,  $\text{Na}_2\text{CO}_3$  and  $\text{CaCl}_2$  mainly cause irrigation, soil salinity, which must be paid for. Soil salinity causes reduced biomass production, which will affect important plant processes (Ahmad & John, 2005; Ahmad & Sharma, 2006; 2008; 2010; Ahmad, 2010; Ahmad et al., 2010 a, b, c, 2012). Öztaş (2018) investigated whether the negative effect of salt stress can be eliminated by applying different doses of  $\text{K}^+$  externally in pepper plants subjected to salt stress, or whether  $\text{K}^+$  will have a stress-reducing effect. It was determined that the same amount of  $\text{K}^+$  ion accumulation was found in

the control at the highest dose. This shows that there was not only a competition between  $\text{Na}^+$  and  $\text{K}^+$  in purchases, but also a serious competition in transportation.

One of the cell parts where oxidative damage caused by stress factors was most effective was cell membranes. ROS are formed in plants under stress cause peroxidation of membrane lipids and cause damage to the cell membrane (Sreenivasulu et al., 2000; Yasar & Ellialtıođlu, 2008), so the resulting ion leakage was determined by the amount of malondialdehyde, a product of lipid peroxidation, which was used as the simplest indicator of damage (Yařar, 2003; Yařar et al., 2006; 2010; Yasar et al, 2008; Yařar & Ellialtıođlu, 2008, Uzal, 2017). High  $\text{Na}^+$  concentration inhibits the uptake of plasmalemma transporters in stem cells. Salinity causes a hormonal imbalance. In addition, it may cause oxidative stress by producing ROS (Azevedo Neto et al., 2008; Tőrkan & Demiral, 2008). Drought stress and salt stress can cause ABA accumulation, followed by stomatal closure and a decrease in carbon dioxide/oxygen content in leaves. It also inhibits  $\text{CO}_2$  fixation. These are a condition for ROS production. Salt stress is more difficult to intervene than other stress factors. The reason for this is that increasing salt concentration changes the ion setting in plants. It affects the water balance necessary for plants. ROS, like other stress factors, can cause oxidative stress by conduction. Adaptation to high salt levels includes osmotic regulation, cleavage of toxic ions, and oxidative stress tolerance. As a result, the response of plants to salt

stress has been documented as dominant in nature (Türkan & Demiral, 2008).

The main factor of soil degradation depends on the salt content of the soil. The salt concentration in the soil affects more than 1/4 of the agricultural lands in the world. The negative effects of salinity on the development of plants; It is known as nutrient imbalance, low water stress in the soil solution, sodium and chloride ion accumulations or combination of these elements. (Munns & Tester 2008). Evelin et al. (2009) reported that soil salinity affects physiological processes such as growth, photosynthesis, protein and lipid metabolism. On top of that, scientists have done a lot of research. Munns & Tester (2008) explained the factors that affect the reduction in growth due to salinity stress. In saline conditions, the plant is malnourished. As a result, the development of the plant is adversely affected. salt stress; It inhibits nutrient uptake, transport within the plant, division or crop rate. Salt stress often impairs food intake in two different ways. First, the ionic strength of the substrate can induce nutrient uptake and displacement. Secondly, salinity creates a competitive environment in the substrate with the main ions sodium<sup>+</sup> chlorine<sup>-</sup> which disrupts the mineral relations of the plants. The interactions that occur mostly lead to chloride inhibition of NO<sup>3-</sup> absorption and sodium<sup>+</sup> induced calcium<sup>+</sup> deficiency (Peuke & Jeschke, 1999). It can cause imbalances and malnutrition by conflicting with the structure and composition of plant cells (Xu et al., 2010).

Salt stress has different effects on plants. This effect varies according to the plant variety and type. The decrease in the water level in the plant causes an increase in sodium and chlorine ions. However, the nutrient transport process of plants under the influence of salt stress is impaired (Munns, 2002). These disorders cause water shortages and nutrient imbalances. In general, plants cannot meet their basic water and nutrient needs under stress. In addition, the increase in osmotic potential and decrease in water content are due to salt stress. They are determinants for the plant's response to salt stress under salt stress. The growth stage and genotype of the plant are taken into account in plants under salt stress. Plants exposed to salt stress have been damaged in various ways. It causes hormonal and enzyme imbalance, antioxidant defense mechanism. It causes disruption of activities such as photosynthesis, respiration, cell membrane dissolution. Serious damage and death occur in the cell (Mahajan & Tuteja, 2005). Consequences of salinity; It affects the inability to germinate, grow, inhibit photosynthesis, change water intake, oxidative stress and decrease yield in plants. Excessive salt concentration in the soil also inhibits nutrient uptake and metabolism.

Plant hormones were classified as special organic substances that act on target tissues at very low concentrations as regulators of growth and development (Kaya et al., 2009). These include ABA, JA, SA and ethylene, which alter the physiological reaction of plants exposed to salt stress. The multiplicity of responses was an important aspect of the complexity of stress signaling. ABA a lipophilic plant hormone was

ubiquitous in lower and upper plants and participates in complex processes throughout the life cycle of plants (Javid et al., 2011).

## 1. AUXINS

Auxins were the first to be discovered in growth regulators and have been used for a long time in agricultural history. These hormones promote growth by increasing the elongation and division of cells. Auxins were synthesized and transported from top to bottom in meristematic tissues such as leaves, apical buds, and flowers. Indole-3-acetic acid (IAA) was the only hormone that can be synthesized naturally in plants. However, it has been determined that many synthetic substances have similar effects to IAA (Kumlay & Eryiğit, 2011). Auxins were examined under 4 groups and the important growth regulators were as follows:

- a) Indole group
  - Indole acetic acid
  - Indole proionic acid
  - Indole butyric acid
- b) Naphthelen group
  - Naphthene Acetic Acid
  - P - Naphthoxy acetic acid
- c) Phenoxy group
  - Phenoxy acetic acid
  - Phenylacetic acid
  - 4 -Chlorophenoxyacetic acid
  - 2,4-Dichlorophenoxy acid
  - 2,4,-5-Trichlorophenoxy acetic acid
- d) Benzole group
  - 2.4.6- Trichlorobe benzoic acid
  - 2.3.6- Trichlorobenzoic acid

- 4- Amino-3,5,6-Trichloropicolinic acid

The effects of these substances on plants were as follows: To promote root formation of cuttings, to provide parthenocarpic fruit set, to provide adventitious root formation, to prevent fruit and leaf fall, to increase fruit set, to enable buds to bloom earlier and to prevent weed growth (Çetin, 2002; Greene, 2006).

Many studies have been carried out to eliminate the inhibitory effect of salt stress on germination by applying plant growth agents. Kaur et al. (1998) determined that IAA decreased the germination percentage and root and stem elongation and fresh and dry weights of the seedlings formed from these seeds, while it was ineffective on  $\alpha$ -amylase activity. On the other hand, studies have been carried out on the germination and growth of wheat seeds. As a result, it has been suggested that the negative effects of salt stress can be alleviated by IAA application (Singh & Darra, 1971; Datta et al., 1997).

## **2. GIBBERELLINS**

Gibberellins are hormones that support growth and development in low doses. GA was discovered in 1926 in the fungus *Gibberella fujikuroi*, which causes over-maturation in rice plants, from which it derives its name. Later, this substance was isolated and named as GA (Vardar, 1970; Kılıç, 2007; Morsünbül et al., 2010). The most widely used gibberellins were GA. Today, it was known that there were at least 126 types of 3 gibberellins. Gibberellins were found in large amounts in buds, embryos, roots, young leaves, flowers, fruits and cambiums of

plants. Among the gibberellins, only GA has commercial importance 3 (Baktir, 2010). The most obvious effect of gibberellins was to increase the elongation of cells. Also; They were very effective in breaking seed and bud dormancy, eliminating stunting, meeting chilling needs, parthenocarpic fruit set and promoting germination (Olszewski et al., 2002; Tyler et al., 2004). In practice, GA<sub>3</sub> was mostly used in table and dried grapes to thin the clusters and increase the grain size.

A large number of studies with seeds of many plant species, the germination percentage of exogenous gibberellic acid (GA<sub>3</sub>) application (Kabar, 1987; Kaur et al., 1998; Khan & Ungar, 2001a) the inhibitory effects of salt stress on seedling growth (Kabar & Baltepe, 1987; Kaur et al., 1998) fresh and dry weight (Datta et al., 1997) and  $\alpha$ -amylase activity (Lin & Kao, 1995; Kaur et al., 1998) proved to be reversed. There was consensus that gibberellins promote the germination of salt-stressed seeds to varying degrees.

Under salt stress, gibberellins cause physiological seeds to go to sleep. It increases water absorption, and stimulates the synthesis and activation of hydrolytic enzymes, especially  $\alpha$ -amylase. It releases sugars and amino acids necessary for embryo development (Ajmal Khan et al., 2004). Salt stress has been shown to significantly reduce  $\alpha$ -amylase activity and germination rate of rice seeds (Liu et al., 2018). It can be alleviated by exogenous GA<sub>3</sub> in rice germination. In addition, they found a positive correlation between bioactive GA content and  $\alpha$ -amylase activity, and between  $\alpha$ -amylase activity and germination rate of rice seeds. On the other hand, gibberellins improve seed germination

by inhibiting ABA activity, either by activating enzymes involved in its catabolism or by blocking the biosynthesis pathway (Miransari & Smith, 2014). In parallel, the application of NaCl-related ABA to okra seeds does not seem to weaken the depressive effect of salinity on the parameters studied. This observation indicates that the inhibitory effect of ABA on germination has already been confirmed by the study of Thakur & Sharma (2005). However, this ABA acts by counteracting the stimulatory effect of GA<sub>3</sub> on them, limiting water absorption and inhibiting the synthesis of germination-specific enzymes such as  $\alpha$ -amylase (Kondhare et al., 2014). The reduction in germination rate observed under ABA treatment can be attributed to secondary dormancy induction and inhibition of seed germination by limiting energy and metabolic availability (Leymarie et al., 2008). Besides GA, ABA also plays an important role in the regulation of seed germination. GA and ABA antagonistically regulate seed germination (Li et al., 2016; Shu et al., 2016), and NaCl, bioactive GA and increased ABA content, which reduces the GA/ABA ratio (Shu et al., 2017). Uzal et al. (2019) in their study, NaCl plus high GA<sub>3</sub> application decreased the level of plant chlorosis and the degree of salt-induced damage. At NaCl plus high GA<sub>3</sub> (10 ppm treatment), plant growth was slowed due to salt-induced stress, but no signs of stress such as curling or chlorosis in plant leaves. All doses of GM treatments reduced antioxidant enzyme activities and MDA content. In summary, application of GA<sub>3</sub> to eggplant partially suppressed the adverse effects of salt stress on plant growth and metabolic activities, and this effect was more pronounced

at a 10 ppm dose of GA<sub>3</sub>. Therefore, GM can provide direct and indirect protection against salt stress results found.

### 3. CYTOKININS

Hormones that initiate cell division. All tissues with active cell division contain high amounts of cytokinins. As the name suggests (cytokinesis = cell division), cytokinins were effective in cell division and function in tissue and organ differentiation (Çetin, 2002). They were usually found in young tissues. It was especially synthesized in root meristems and then transported to the green parts of the plant via xylem. While auxins promote root formation, cytokinins promote shoot formation. They contribute to organ formation and development in tissue culture environments (Güleryüz, 1982; Kumlay & Eryiğit, 2011). The first plant-based cytokinin was zeatin isolated from maize seeds. Zeatin, dihydrozeatin, isopentenyl adenine (2IP) and dimethylallyladenine were naturally synthesized cytokinins, besides kinetin (N<sup>6</sup> furfurylamino purine), benzyladenine (BA) and tetrahydropyranlybenzyl adenine (PBA) were synthetic cytokinins (Unsal, 1993). Benzyladenine was the most commonly used cytokinin in applications. Thidiazuron (TDZ), which has been discovered in recent years, was also a highly effective cytokinin (Baktır, 2010). More than thirty cytokinins have now been isolated.

It has been found that cytokinins can alleviate the negative effects of salt stress on seed germination (Khan & Ungar, 2001a, c; Gulzar & Khan, 2002) and were ineffective in some cases (Khan & Ungar, 2001b). In addition to the application of GA<sub>3</sub> and cytokinin alone, there

were many data that the combination applications of these two regulators were quite successful in eliminating the negative effects of salt on seed germination (Kabar & Baltepe, 1987). It has also been found that Kin promotes seedling growth under saline conditions (Datta et al., 1997; Kaur et al., 1998). It was known that the amount of cytokinins and gibberellins decreases in response to the increase in the intrinsic amount of ABA in various plant tissues under salt stress (Itai, 1978; Bozcuk & Topçuoğlu, 1982; Lerner, 1985). Therefore, it was found logical that the use of these two stimulatory regulators separately and together could alleviate the salt stress during seed germination. On the other hand, it has also been suggested that gibberellins may need the help of cytokinins, especially under high salt stress conditions (Khan, 1971; Kabar & Baltepe, 1987).

#### **4. ABSCISIC ACID (ABA)**

In addition to natural substances that promote growth in plants, there were also inhibitory natural substances that act in the opposite direction. The most important of these substances was abscisic acid (ABA) (Morsünbül et al., 2010). It was a natural antagonist of auxin, gibberellins and cytokinins, known as ABA-promoting hormones (Kumlay & Eryiğit, 2011). ABA was found in every organ of plants. However, it was mostly synthesized in the cytoplasm of leaf mesophyll cells and found in green leaves (Baktır, 2010). ABA was not synthesized in the roots because there were no chloroplasts in the roots. It is thought that it was found in high amounts in dormant buds and seeds and causes dormancy to continue (Kumlay & Eryiğit, 2011).

ABA concentration in plants changes depending on environmental conditions, and its effect on physiological events also changes. The amount of ABA synthesized under stress conditions increases and was rapidly transported to the petiole and stem tissues and to other parts of the plant (Özen & Önay, 1999). The amount of ABA increases during water stress in plants, causing the closure of stomata and slowing down protein synthesis. In addition, ABA acts as an inhibitory substance in the dormancy of storage organs such as seeds, buds and tubers and ensures the storage of protein synthesis in seeds (Çetin, 2002; Seçer, 1989). it has no usage area (Davies, 1995).

Salinity, drought and cold stress cause abscisic acid (ABA) biosynthesis and accumulation (Borsani et al., 2003). Salt stress in *Citrus sinensis* increases ABA and ethylene production. ABA was responsible for promoting various genes under salt stress. ABA-inducible genes are involved in the salt tolerance mechanism in rice plants. ABA was involved in the transport of growth-development, photosynthesis and assimilation products. At the same time, ABA reduces the inhibitory effect of NaCl. In addition, ABA initiates the conversion from C3 to CAM in the *Mesembryanthemum crystallinum* species. Under stress conditions, ABA initiates stomatal closure by rapidly reducing ion flow in stomatal guard cells (Parida & Das, 2005). Under salt and other environmental stresses, the ABA concentration in leaves can increase 50 times. As a result of the accumulation of ABA in the leaves, the closure of the stomata occurs to reduce water loss (Bressan, 2008). However, there was evidence that ABA was required in the control of

ion balance. The uptake and accumulation of  $K^+$  from plant roots has been shown to be controlled by ABA. The accumulation of  $Ca^{2+}$  in the cytoplasm, which was important in ion balance, was promoted by ABA (Borsani et al., 2003).

Hormones act as regulators of plants against abiotic stress. ABA is a key regulator especially against osmotic stresses (Hubbard et al., 2010; Kim et al., 2010; Chinnusamy et al., 2008). It has rapid signaling and transcriptional activity (Cramer et al., 2011). ABA activates a number of responses that result in increased tolerance to dehydration stress. As a result of scientific studies, it has paved the way for the activation of both ABA-dependent and ABA-independent pathways during dehydration and salinity stresses.

Abscisic acid was a very important plant hormone that regulates different plant activities under different conditions, including stress. Under stress, ABA results in the production of  $H_2O_2$ , which as a signal can mediate the plant's response to stress.  $H_2O_2$  was involved in different plant activities, including root growth and development, evapotranspiration, cell cycle, plant-microbe interactions, and plant response to stress.

## **5. OTHER HORMONES**

In addition to the main 5 hormone groups that affect growth and development, there were various growth regulators. These were brassinosteroids, jasmonates, salicylic acid and polyamines.

Plant hormones were very important for different plant activities and development. The plant's response to stress and hence plant immunity. (SA), (JA) and (ET) were important hormones for plant immunity when interacting with different microbes such as pathogens (Spoel & Dong 2008). Pathogenic microbes can alter plant hormonal homeostasis. For example, Cui et al. (2010) noted that AvrB protein production by *Pseudomonas syringae* may affect plant hormonal signaling, such as upregulation of JA response genes, resulting in increased plant susceptibility. However, *Arabidopsis* MPK4, chaperone components, and interacting proteins were required for the AvrB protein to act effectively. The interaction between such parameters results in activation of the AvrB signaling pathway and hence the subsequent alteration of plant hormonal signaling and increased plant susceptibility to the benefit of bacteria.

### **5.1. Brassinosteroids**

Brassinosteroids (BR) were first described in pure form in 1979 by Grove et al. It was isolated from the pollen of the rapeseed (*Brassica napus*) plant. Brassinosteroids in the steroid structure were named after the *Brassica* genus of the Cabbage family (Cruciferae). Although the synthesis of BRs was not fully explained today, the starting material was mevalonic acid. BRs were hormones that were very effective in plant stem elongation. Exogenously applied BR to some dwarf mutant plants that cannot produce steroids significantly increases stem elongation. Likewise, when BR was applied to dwarf beans, height increases as a result of cell division and cell elongation increase (Baktir,

2010; Clouse, 2009). BRs were also used for many purposes such as providing tolerance to salt stress, cold and disease-pests, preventing fruit set, increasing yield, promoting germination and promoting root growth (Rao et al., 2002).

## **5.2. Jasmonates**

Firstly, jasmonates obtained from jasmine (*Jasminum grandiflorum*) plant include esters of jasmonic acid (JA) and methyl jasmonate (MeJA) (Fan et al., 1998). Jasmonates were synthesized by flowers, leaves, roots and immature fruits (Baktır, 2010). Jasmonates were especially effective in the resistance mechanisms of plants and increase the resistance against diseases and pests. When Jasmonates were applied externally; It has been determined that it inhibits photosynthesis, pollen germination, longitudinal development, growth in roots, formation of flower buds, germination of seeds whose embryogenesis was not at rest. Besides the inhibitory properties of jasmonates, there were also encouraging aspects; It promotes adventitious root formation, abscission, closure of stomata, protein synthesis, resting need, germination of resting seeds and ethylene synthesis, thus fruit ripening (Fan et al., 1997; Yıldız & Yılmaz, 2001). In a study conducted by Uzal (2009), the effect of jasmonic acid (JA) on the salt tolerance mechanism was investigated. While NaCl application alone caused a significant increase in leaf maleondialdehyde (MDA) content, this increase was not observed when NaCl was applied together with JA. That is, JA inhibited the effect of salt on leaf MDA content. Compared with control and NaCl

applications, NaCl<sup>+</sup> JA applications caused significant increases in APX and CAT activities. On the other hand, this increase was not observed in plants where JA was applied alone.

### **5.3.Salicylic Acid**

Salicylic acid (SA) is named after the willow (*Salix*) tree, whose leaves and bark have been known for centuries to be good for pain and fever. Salicylic acid (SA), usually a hydroxyl group or its plant with an aromatic ring bearing a functional derivative. It is a group of phenolics. In 1828, Johann Buchner isolated it from willow bark in Germany. The name salicylic acid was first used in 1838 by a researcher named Rafaele Piria. Immediately after, scientific studies on SA were tried to be done. The works are still in progress. Commercial production of salicylic acid took place in Germany under the name 'Aspirin'. In recent years, studies on the effect of SA on plants have shown that it is quite effective in the regulation and improvement of plant growth. The first studies have been on how SA is affected in which organs or parts of the plant. Salicylic acid is found in all organs of the plant and is transported to different organs via phloem from the place where it is applied externally (Baktır, 2010). The effect of salicylic acid on flowering was investigated. It has been determined that the aspirin tablet prolongs the vase life of cut flowers (Özeker, 2005). The effects of salicylic acid on plants have been investigated by scientific studies. It has been found to block ethylene synthesis in apples. It increases grain yield in beans. There have been many studies showing that it increases rooting and accelerates photosynthesis (Hayat et al., 2007; Ramanujam et al., 1998;

Romani et al., 1989). The most effective usage areas of SA have generally been on abiotic stress factors. Some of these have been aimed at increasing resistance to conditions such as drought, salinity, high and low temperatures, heavy metals and frost stress (Baktir, 2010). Phytohormones are crucial for abiotic stress tolerance such as salicylic acid (SA) which is involved in the regulation of acquisition and assimilation of nutrient elements including S in plants under stress conditions (Wang et al., 2011; Khan et al., 2015). The role of SA in defense mechanism to alleviate salt stress in plants was studied (Afzal et al., 2006; Eraslan et al., 2007; Hussein et al., 2007). The mitigation effect of SA to abiotic stresses was investigated through its application either by foliar spray of maize (Khodary, 2004), seed soaking of wheat genotypes (Al-Hakimi, 2006) or through rooting medium of wheat (Arfan et al., 2007). The effect of salicylic acid on the physiological processes is variable, promoting some processes and inhibiting others depending on its concentration, plant species and environmental conditions (El-Mergawi & Abdel Wahed, 2004)

### **5.3.Polyamines**

Among the polyamines; Diamine, putrescine, triamine, spermidine, and tetramine were ubiquitous in plant cells of sperm (Smith, 1970; Bagni & Pistocchi, 1992). The most important polyamines are putrescine, spermidine and spermine. Polyamines are involved in events such as cell division, root formation and incidental shoot formation in tissue culture.

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## CHAPTER X

### ROOTING CHARACTERISTICS OF SIX DIFFERENT CULTIVARS OF *Lavandula angustifolia* CUTTINGS

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## 1. INTRODUCTION

Lavender is native to the mountainous regions of the Mediterranean, but today it is widely cultivated in Europe, Australia, the United States, and Bulgaria (Kara, 2011, Karakaş & İzci 2021). There are about 39 lavender species (*Lavandula* sp.), most of which are of Mediterranean origin, and there are three main lavender species with high commercial value in the world. English lavender (*Lavandula angustifolia* Mill) and Spike lavender (*Lavandula spica* = *L. latifolia* Medik.) have higher essential oil quality, while the hybrid named Lavandin (*Lavandula intermedia* Emeric ex Loisel. = *L. hybrida* L.) has a higher essential oil yield (Tucker, 1985). Apart from these three important species, *L. dentata* (French), *L. stoechas* L., *L. latifolia* Medik, *L. multifida*, *L. canariensis*, *L. lanata*, *L. heterophylla* and *L. x allardii* (*L. dentata* x *L. latifolia* Medik.) is mostly used for ornamental or decorative purposes, (Kara & Baydar, 2013).

Lavender, known as the essential oil plant, is a semi-shrub, perennial, aromatic plant in the Lamiaceae family (Aslanca & Saribaş, 2011). It is produced for the essential oil obtained from the flowers and herbs of the plant. In addition, lavender is used as an ornamental plant in landscaping, beekeeping and eco tourism. Lavender essential oil is generally used in cosmetics, perfumery, medicine and soap industries.

Lavender reproduces vegetatively (with cuttings) and generatively (with seeds), and providing propagation material with high yield and quality is the most important problem. *Lavandula* species are propagated by seeds but it is not a preferred method for its propagation

because the method is resulting in a great lack of uniformity. Also, asexually propagated lavender crops yield more homogeneous crops so high-quality clones increase the likelihood of obtaining higher-quality essential oil (Tyub et al., 2007). Therefore, especially in recent years, the increasing interest in lavender agriculture has increased the importance of scientific research on advanced cultivation techniques of this plant and significant developments have been made. However, there are some problems in propagation with cuttings in lavender. Low rooting is the most important of these problems and to solve this problem, cutting types and different rooting media are studied (Bona et al., 2012a).

In recent years, the popularity of lavender has been increasing in Turkey due to its rich essential oil content. This situation also increases the interest in lavender seedling production. However, it is necessary to grow plants in a certain standard in order to carry out lavender seedling production in Turkey in accordance with the domestic and foreign market. This is only possible by producing propagation material (seedling) with high yield and quality. Seedling needed in lavender can be produced in two ways; by seed or by cutting. Since lavender is a foreign pollinated plant, yield and quality cannot be maintained in seedling production with seeds. By preserving the yield and quality of the rootstock, seedling production can only be obtain with cuttings.

In propagation by cuttings, issues such as cutting type, cutting time and dosage of hormones that support rooting and shoot development should be determined according to plant species and ecological conditions

(Putievsky et al., 1983; Nicola et al., 2003; Kara et al., 2011). Indeed, in some studies showed that the rooting rate of cutting varies depending on the cutting type and rooting environment (Beatovic et al., 2012; Bona et al., 2012a; Özcan et al., 2013), some hormones such as IBA, NAA and levels, and waiting times (Ayanoğlu et al., 2002; Bhat et al., 2008; Bona et al., 2010; Bona et al., 2012b, Arslanoğlu & Albayrak, 2011).

## 2. MATERIAL AND METHODS

In the study, the cuttings of Hemus, Tiruzihaba, Yalanka, Raya, Hebar, Sevtopolis consisting of six cultivars belonging to the *Lavandula angustifolia* species, were taken from the Tekirdağ Research Station on January 28, 2020. The study was carried out on the rooting unit in the plastic greenhouse of Department of Horticulture, Faculty of Agriculture, Hatay Mustafa Kemal University. In the experiment, perlite was used as rooting medium and the cuttings were planted with three replications and 15 cuttings in each replication.

Herbaceous cuttings used in this work. Cuttings were prepared using a bevel cut just below a node, discarding the leaves at the basal part, and keeping two pairs of leaves in the upper part which were later cut in half. During planting, the cuttings were kept in a plastic bag containing water to avoid dehydration. The cuttings with a length of 15 cm were used in the study.

The cuttings were arranged in a mist unit with an intermittent misting regimen controlled by a timer and solenoid valve. The valve was

programmed to mist for 15 sec every 12 hour . The mist propagation unit was placed in a greenhouse with a transparent polyethylene film.

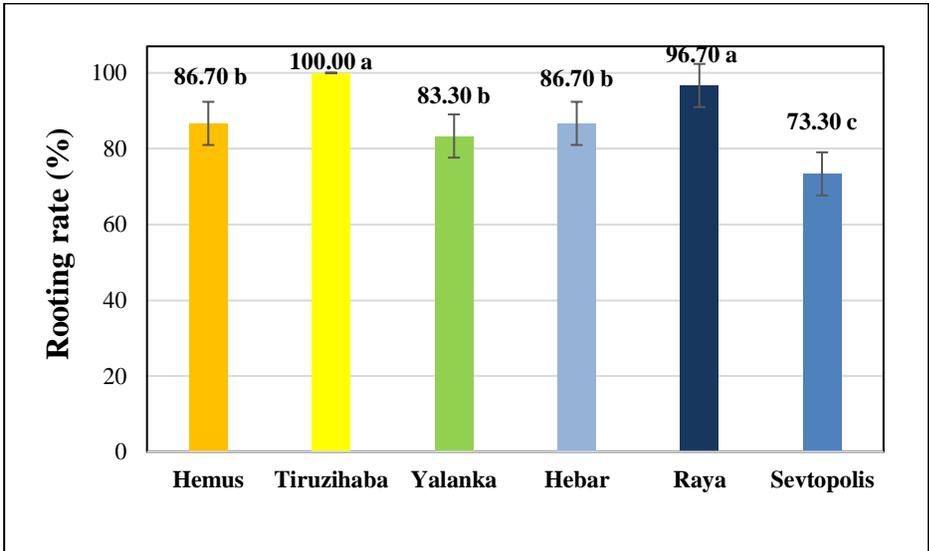
Rooting rate (%), root length (cm), root number (number), shoot number (number) and shoot length (cm) of cuttings were investigated 60 days after planting. Data Analysis of variance was carried out according to random plots experimental design and the mean values compared by Tukey's honestly significant difference test (HSD) using the SAS package program (SAS, 2005).

### **3. RESULTS AND DISCUSSION**

#### **3.1. Rooting Rate (%)**

Differences in rooting rate of cuttings belonging to lavender cultivars were statistically significant at the  $P \leq 0.05$  level. In the study, the highest rooting percentage was found in Tiruzihaba (100%) and Raya (96.7) cultivars. The lowest rooting rate was found in Sevtopolis cultivar with 73.3% (Figure 1). Our findings was similar to those obtained by Ayanoğlu et al. (2000), who indicated that *Lavandula stoecha* cuttings of the highest rooting 4000 ppm IBA application (70%). Similarly, Bhat et al. (2008) showed that the highest rooting rate in *Lavandula officinalis* was 3000 ppm IBA application (90.3%). Özcan et al. (2013) reported that rooting rate of *Lavandula hybrida* cuttings was the highest at 2000 ppm IBA (87.50%) application. These results were also in agreement with previous studies that showed that Rooting rate in cuttings varied according to lavender species and cultivars (Kara and Baydar, 2020; Karakaş and Izci, 2021). However, Swetha (2005)

displayed that type of cutting, rooting medium, cutting taking season, preliminary applications, and environmental conditions can be affect rooting success in lavender.



**Figure 1:** Effect of Different *Lavandula angustifolia* Cultivars on Rooting Percentage

### 3.2. Number of Roots

The highest root number was determined in Tiruzihaba cultivar (86.7), while the lowest root number was determined in Sevtopolis cultivar (7.6) (Table 5). Bhat et al. (2008) reported that the mean root length in *Lavandula officinalis* was 10.30 cm. Bona et al. (2010) showed that the average root number was 38 in *Lavandula dentata* cuttings. Also, the mean root numbers was 17.44 in *Lavandula angustifolia* Mill (İzgi 2020) and 3.16 in *L. angustifolia* var. Raya and 1.85 in *L.x intermedia* var. Super (Kara and Baydar, 2020). The differences may be due to the genotype and environment conditions.

### **3.2. Root Length (cm)**

Hemus cultivar had the longest root length (8.4 cm), whereas Sevtopolis had the shortest root length (5.6 cm) (Table 5). In previous studies, root length values ranged from 10.30 cm to 24.80 cm in *Lavandula officinalis* (Bhat et al., 2008), ranged from 7.69 cm and 10.21 cm in *Lavandula dentata* (Bona et al., 2010), and ranged from 17.04 cm and 25.10 cm in *Lavandula angustifolia* (İzgi, 2020). Also, Karakaş & İzci (2021) reported that root length was 2.66 cm in of Hemus cultivar and 3.32 cm in Sevtopolis cultivar.

### **3.3. Number of Shoots (Number)**

The number of shoots on the cuttings were shown in Table 1. The highest number of shoots was determined in Tiruzihaba cultivar (3.8 units). The lowest number of shoots was detected in Raya cultivar (1.6 units). Similarly, Çiçek & Özel (2021) indicated that the number of shoots per cutting in *Lavandula angustifolia* Mill. ranged from 1.66 to 4.68. In addition, Karakaş & İzci (2021) showed that the number of shoots per cutting was 7.60 in Hemus cultivar and 7.70 in Sevtopolis cultivar.

### **3.4. Shoot Length (cm)**

The highest shoot length is 3.90 cm in Hemus cultivar, whereas it was the lowest in the Tiruzihaba cultivar (2 cm) (Table 1, Figure 2).

**Table 1:** Effect of Different *Lavandula angustifolia* Cultivars on Rooting

Genotypes	Root Number (number)	Root Length (cm)	Shoot Number (number)	Shoot Length (cm)
Hemus	49.20 b	8.40 a	2.10 bc	3.90 a
Tiruzihaba	86.70 a	6.60 ab	3.80 a	2.00 c
Yalanka	14.00 bc	5.70 ab	2.50 b	2.70 bc
Hebar	8.90 bc	6.00 ab	2.30 bc	3.20 ab
Raya	15.10 bc	7.30 ab	1.60 c	3.40 ab
Sevtopolis	7.60 c	5.60 b	1.80 bc	2.50 bc



**Figure 2:** Rooting Images of Different *Lavandula angustifolia* Cuttings (Hemus, Tiruzihaba, Yalanka, Hebar, Raya, Sevtopolis)

These results were in agreement with those of Çiçek & Özel (2021), who showed that shoot length in cuttings varied between 3.54 cm and 4.87 cm depending on cutting types in *Lavandula angustifolia* Mill. In addition, Karakaş & (2021) reported that shoot length in cuttings was 4.75 cm in Hemus cultivar and 5.90 cm in Sevtopolis cultivar.

#### 4. CONCLUSION

In recent years, there has been an increasing interest in lavender culture in Turkey. Lavender, which is a dry agricultural plant that is very well adapted to barren and sloping areas in Turkey. For this purpose, the lavender cultivars that produce essential oil of marketable quality in the world can be cultivate in these areas. However, the seedlings of these cultivars should be rapidly propagated and distributed to the producers.

In our study with six different *Lavandula angustifolia* cultivars, it was determined that all cultivars could be rooted without using IBA. When the rooting rates of different cultivars were compared with cuttings, the best results in terms of rooting rate and root characteristics were obtained from Tiruzihaba cultivar for lavender type. This cultivar was followed by Raya cultivar for the same species. The variety showing the weakest rooting characteristics was Sevtopolis. Although the cuttings were rooted without using any growth regulator or hormone in the cultivars used in this study, the rooting rates were quite high in the lavender species.

Rooting of cuttings in many species is either very slow or non-existent. In hard-to-root species, rooting is provided or root formation is accelerated with the help of auxin group hormones. However, despite the use of hormones from various sources in lavender, the most important gain determined by this study is that the cuttings can be rooted quickly and uniformly without hormone applications.

In terms of cutting periods, it should be examined how the lavender varieties will show rooting. Another important issue may be the determination of the changes in the internal hormones, enzymes, phenolic compounds and total sugar ratios in the cuttings. In this way, the reason for the low rooting rate can be explained more clearly in the light of physiological events.

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## CHAPTER XI

### THE RESPONSES OF SEEDLING GROWTH AND NUTRIENT CONTENTS TO PEAT APPLICATION IN HEAVY METAL CONTAMINATED GROWING MEDIA

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## 1. INTRODUCTION

Heavy metal contamination in agricultural soils has attracted worldwide attention due to their adverse effects on soil quality and food security (Adimalla et al., 2019; Antoniadis et al., 2019; Sonne et al., 2020). Large amounts of various heavy metals are released into the environment due to anthropogenic and industrial activities. These metals threaten human health by contaminating the food chain and other sources (Nagarajan et al., 2020).

It has been reported that heavy metals cause toxicity in living things due to their toxic and non-biodegradable structures (Adrees et al., 2015, Yousaf et al., 2016).

Cadmium within the toxic heavy metals was reported as one of the most hazardous elements in environment (Nagajyoti et al., 2018; Rizwan et al., 2018). Phosphate fertilization, raw industrial and municipal effluents, sewage sludges, mining waste incineration and atmospheric deposition cause Cd accumulation in agricultural lands (Murtaza et al., 2015; Qayyum et al., 2017). Rizwan et al. (2017) reported that Cd causes stunted growth, chlorosis and browning in roots, and a decrease in photosynthesis, depending on its concentrations in the plant growing medium.

It has also been reported that Cd causes the accumulation of reactive oxygen species (ROS) in plants and, as a result, various metabolic dysfunctions in plants (Rizwan et al., 2016a).

The hydroponics, pots and field applications are among a large number of techniques considered for the reduction of heavy metal uptake by numerous plant species at various levels (Ali et al., 2015; Khaliq et al., 2016; Yousaf et al., 2017). It was reported that various organic amendments, among several amendments, are previously used by purpose to decrease the metal uptake by plants and enhancing soil properties (Bian et al., 2014; Rehman et al., 2017).

The composts, organic carbon, tree bark, sawdust, green manure, sewage sludge, and peat which are various organic soil amending substances can be used for remediation of soil polluted with heavy metals. The additions of this materials into soil reduces amounts of mobile forms of metals in soil (Kumpiene et al., 2007; Gondek, 2009). Wrobel & Nowak-Winiarska (2011) reported that organic materials added to soil inhibits uptake of heavy metals by plants. Organic soil conditioners have been preferred over inorganic ones due to their higher biodegradability and environmental friendliness and many more numerous benefits (Rizwan et al., 2016b).

Krogstad (1983) reported that organic matter makes strong complexes with heavy metals. Soil organic matter may retain metals in the solid phase of the soil, on the contrary dissolved organic matter may increase mobility of the metals (Japenga et al., 1992; Lo et al., 1992). The availability for uptake by plant roots may differ between metals bound in soluble organic complexes and free metals. It was reported that organic materials influence the binding of heavy metals in soil and speciation

in soil solution (Lo et al., 1992; Dell Castillho et al., 1993) and plant uptake (Haghiri 1974; Mc Bride et al., 1981).

Among various organic amendments peat have different mechanisms in the sorption of metals. These mechanisms changes depend on the type of peat and metal, metal concentration. The pH value has a key role in sorption of metal ions by peat from water. The range of 3.5 -6.5 is optimum pH value for metal sorption (Brawn et al., 2000).

Some researchers reported that the ion-exchange mechanism is most widespread among aforementioned mechanisms (Chen et al., 1990; Ho et al., 1995; Crist et al., 1996). Metal adsorption of peat happens by complexing, surface adsorption by chemisorption (Sharma & Forster, 1993).

## **2. MATERIAL AND METHODS**

In this study, three mixtures of garden soil: peat (P<sub>0</sub>: control (%100 soil), P<sub>1</sub>:10% peat and P<sub>2</sub>:20% peat) were used as plant growing media. This study was conducted with three different levels of Cd (Cd<sub>0</sub>; 0, Cd<sub>1</sub>; 2.5 mg kg<sup>-1</sup>, Cd<sub>2</sub>; 5.0 mg kg<sup>-1</sup>) as Cd(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O and three different ratios of peat in the growing media in a factorial design with three replications. The garden soil used in the study had a sandy loamy texture, non-saline, alkaline, moderate in lime and organic matter contents, insufficient in phosphorus and sufficient in potassium content. The peat used in the study had non-saline, slightly alkaline, high in organic matter, phosphorus, and potassium contents.

**Table 1:** Some Properties of the Growing Medias

	Texture	pH	Total Salinity	Lime	OM	N	P	K
				%				mg kg <sup>-1</sup>
Soil	Sandy Loam	8.66	0.013	14.8	2.40	0.192	4.56	176
Soil+10% Peat	-	8.54	0.022	9.38	27.9	0.210	5.67	184
Soil+20% Peat	-	8.37	0.026	8.38	30.2	0.215	6.78	287
Peat	-	7.76	0.030	-	69.8	0.311	25.67	495

OM; Organic Matter, N; Nitrogen, P; Phosphorus, K; Potassium

After filling each pot (300 cm<sup>3</sup>) without drainage holes with soil: peat mixtures, 270 pots were autoclaved. Each replication was formed from ten pots. As a basic fertilizer treatment 90 mg kg<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>, 180 mg kg<sup>-1</sup> K<sub>2</sub>O and 250 mg kg<sup>-1</sup> N were also applied into each pot from Triple Super Phosphate (%44 P<sub>2</sub>O<sub>5</sub>), K<sub>2</sub>SO<sub>4</sub> (%50 K<sub>2</sub>O) and (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> (%21 N), respectively. Demre pepper variety was used as a plant material. Three pepper seeds were sown to each pot, and then the seedling was thinned to one. Experiment was carried out in a plant growth room of Department of Horticulture in Yüzüncü Yıl University under controlled conditions. The pots were placed in a growth chamber at 22±1 °C with 12 fluorescent illuminations with 8000 lux light intensity and the seedlings were irrigated with distilled water. The experiment was ended 8 weeks after the sowing.

The levels of nutrients were analysed in dried and grinded samples according to the methods reported by Kacar & İnal (2008). Phosphorus level was analyzed by spectrophotometric method, Ca, Mg, Fe, Mn, Zn, and Cu levels were determined by atomic absorption spectrophotometers.

Variance analyses of the experimental data were done by TARIST statistic program and significantly different means numbered according to LSD test.

## 2. RESULTS AND DISCUSSION

Variance analyses results for the seedling criterias are given in Table 2. Shoot fresh weight, leaf number, shoot length, root fresh weight, and root length were significantly ( $P < 0.01$ ) influenced by the different ratios of peat treatment. Interactions of peat and cadmium significantly affected root fresh ( $P < 0.01$ ) and root length ( $P < 0.05$ ).

**Table 2:** F Values of the Variance Analyses for the Seedling Criterias

Variance	DF	Shoot fresh weight	Leaf number	Shoot length	Root fresh weight	Root Length
Peat (Pt)	2	206.82 **	64.47 **	36.43 **	65.32 **	17.53 **
Cadmium	2	0.07 ns	0.24 ns	2.43 ns	1.12 ns	0.94 ns
Pt x Cd	4	0.56 ns	2.41 ns	1.06 ns	26.74 **	3.16 *

\*\* significant at 0.01 level, \*significant at 0.05 level, ns: non-significant

Application of 20% peat ratio significantly increased shoot fresh and dry weights, leaf number, shoot length and root fresh weight compared to control and 10% peat ratio (Table 3). While the highest mean shoots fresh weight, leaf number, shoot length and root fresh weight were obtained as 17.31 g, 9.78 cm, 9.10 cm and 6.87 g in the application of 20% peat ratio respectively, the lowest means in these parameters were in the application of 10% peat ratio (Table 3).

The highest mean root length was determined in growth media including mixture of garden soil:no peat as 12.50 cm respectively. The

cadmium doses decreased root fresh and and root length. But these decreases were found non-significant statistically (Table 3). When the interactions between garden soil:peat ratio and cadmium doses were considered, the highest mean root fresh weight was determined in Pt<sub>20</sub>Cd<sub>0</sub> application while the highest root length was in Pt<sub>0</sub>Cd<sub>0</sub> interaction (Figure 1).

**Table 3:** Effects of Peat and Cadmium Applications on the Pepper Seedling Criterias

Treatments	Shoot Fresh Weight	Root Fresh Weight	Shoot Length	Root Length	Leaf Number
	g		cm		No.
<b>Cd, mg kg<sup>-1</sup></b>					
0	11.248	6.00	7.97	10.82	8.47
2.5	11.312	5.72	8.37	10.62	8.41
5.0	11.442	5.67	7.82	10.03	8.37
LSD (.05)	1.129	0.67	0.54	1.73	0.47
<b>Peat, %</b>					
0	10.112 b	6.22 a	8.16 b	12.50 a	8.22 b
10	6.589 c	4.30 b	6.90 c	9.11 b	7.25 c
20	17.302 a	6.87 a	9.09 a	9.85 b	9.78 a
LSD (.05)	1.129	0.67	0.54	1.73	0.47

Mean values with different lower-case letters among columns are significantly different at  $p < 0.05$



**Figure 1:** Effects of Peat x Cadmium Interactions on the Pepper Seedling Criterias

Variance analyses results for the cadmium and nutrient contents of shoots were given in Table 4. The peat applications significantly affected Ca, Mg, P, Cu, Zn, and Mn contents of shoots ( $P < 0.01$ ). The cadmium doses also significantly influenced Mg, Zn, Mn, Cu ( $P < 0.01$ ) and Fe, P ( $P < 0.05$ ) contents of shoots.

**Table 4:** F Values of the Variance Analyses for the Cadmium and Nutrient Contents of Shoots

Variance	DF	Ca	Mg	P	Fe	Zn	Mn	Cu
Peat (Pt)	2	31.23**	39.11**	53.14 **	18.17 **	287.25 **	132.10 **	29.90**
Cadmium	2	0.905 ns	7.36**	5.84 *	3.01 ns	13.73 **	15.25 **	24.25 **
Pt x Cd	4	0.241 ns	12.62**	6.76 **	1.18 ns	2.78 ns	16.75 **	27.47 **

\*\* significant at 0.01 level, \*significant at 0.05 level, ns: non-significant

The effects of interactions between peat ratio and cadmium doses were found as significant ( $P < 0.01$ ) for Mg, P, Mn, Cu contents of shoots (Table 4).

Variance analyses results for the cadmium and nutrient contents of roots were given Table 6. Peat applications affected all nutrient contents of roots analyzed were significantly ( $P < 0.01$ ). Cd, Fe, Mn, Zn, and Cu contents were significantly ( $P < 0.01$ ) influenced by the increasing Cd doses. Effects of interactions between peat and cadmium were found as significant for Ca ( $P < 0.05$ ), P, Fe, Mn, and Cu ( $P < 0.01$ ) contents of roots (Table 5).

**Table 5:** F Values of the Variance Analyses for the Cadmium and Nutrient Contents of Roots

Variance	DF	Ca	Mg	P	Fe	Zn	Mn	Cu
Peat (Pt)	2	19.06 **	7.14 **	1.56 ns	61.05 **	37.91 **	273.28 **	44.11 **
Cadmium	2	0.11 ns	0.48 ns	0.14 ns	10.45 **	14.74 **	15.92 **	21.19 **
Pt x Cd	4	3.61 *	0.94 ns	4.83 **	4.72 **	3.21 *	12.51 **	5.69 **

\*\* significant at 0.01 level, \*significant at 0.05 level, ns: non-significant

Effects of peat and cadmium applications on the cadmium and nutrient contents of shoots and comparison of the means according to LSD test are given in Table 6 and 7. Application of 20% peat ratio (Pt<sub>20</sub>) increased P (2210 mg kg<sup>-1</sup>), Fe (314 mg kg<sup>-1</sup>) and Zn (9.58 mg kg<sup>-1</sup>) contents of shoots compared with mixtures including no peat and 10% peat (Table 6, Table 7).

While the P, Fe, Cu contents were increased by the Cd applications, Mn, and Zn contents were diminished compare with Cd<sub>0</sub> application (Table 6, Table 7). The highest means of Mn and Zn contents were

obtained in Cd<sub>0</sub> application as 135 mg kg<sup>-1</sup>, and 6.57 mg kg<sup>-1</sup> respectively (Table 7). When peat and cadmium interaction is noticed, the highest means of Mg and Cu contents were in Pt<sub>0</sub>Cd<sub>2</sub> and the highest means of P content was in Pt<sub>20</sub>Cd<sub>2</sub> (Figure 2).

**Table 6:** Effects of Peat and Cadmium Applications on the Nutrient Contents of Shoots and Root

Treatments	Ca, %		Mg, %		P, mg kg <sup>-1</sup>	
	Shoot	Root	Shoot	Root	Shoot	Root
<b>Cd, mg kg<sup>-1</sup></b>						
0	1.567	0.712	0.695 b	0.0895	1487 b	3629
2.5	1.528	0.742	0.743 b	0.0848	1485 b	3685
5.0	1.442	0.737	0.895 a	0.0763	1809 a	3721
<b>Peat, %</b>						
0	1.210 b	0.506 c	0.952 a	0.0971 a	1461 b	3736 ab
10	1.929 a	0.760 b	0.877a	0.0881 a	1110 c	3796 a
20	1.397 b	0.925 a	0.504 b	0.0609 b	2210 a	3504 b

Mean values with different lower-case letters among columns are significantly different at p< 0.05

**Table 7:** Effects of Peat and Cadmium Applications on the Nutrient Contents of Shoots and Root

Treatments	Fe, mg kg <sup>-1</sup>		Zn, mg kg <sup>-1</sup>		Mn, mg kg <sup>-1</sup>		Cu, mg kg <sup>-1</sup>	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
<b>Cd, mg kg<sup>-1</sup></b>								
0	246 a	5645 a	6.57 a	26.36 a	135 a	357 a	18.47 a	29.44 c
2.5	230 ab	5361 a	5.21 b	22.10 b	116 b	313 b	11.68 b	34.64 b
5.0	211 b	4739 b	5.28 b	18.51 b	109 b	296 b	12.58 b	38.88 a
<b>Peat, %</b>								
0	182 b	6174 a	4.66 b	29.79 a	152 a	458 a	12.78 b	28.09 c
10	239 a	5568 b	2.82 c	19.71 b	132 b	311 b	11b91 b	33.27 b
20	266 a	4003 c	9.58 a	17.47 b	77 c	1978 c	18.04 a	41.61 a

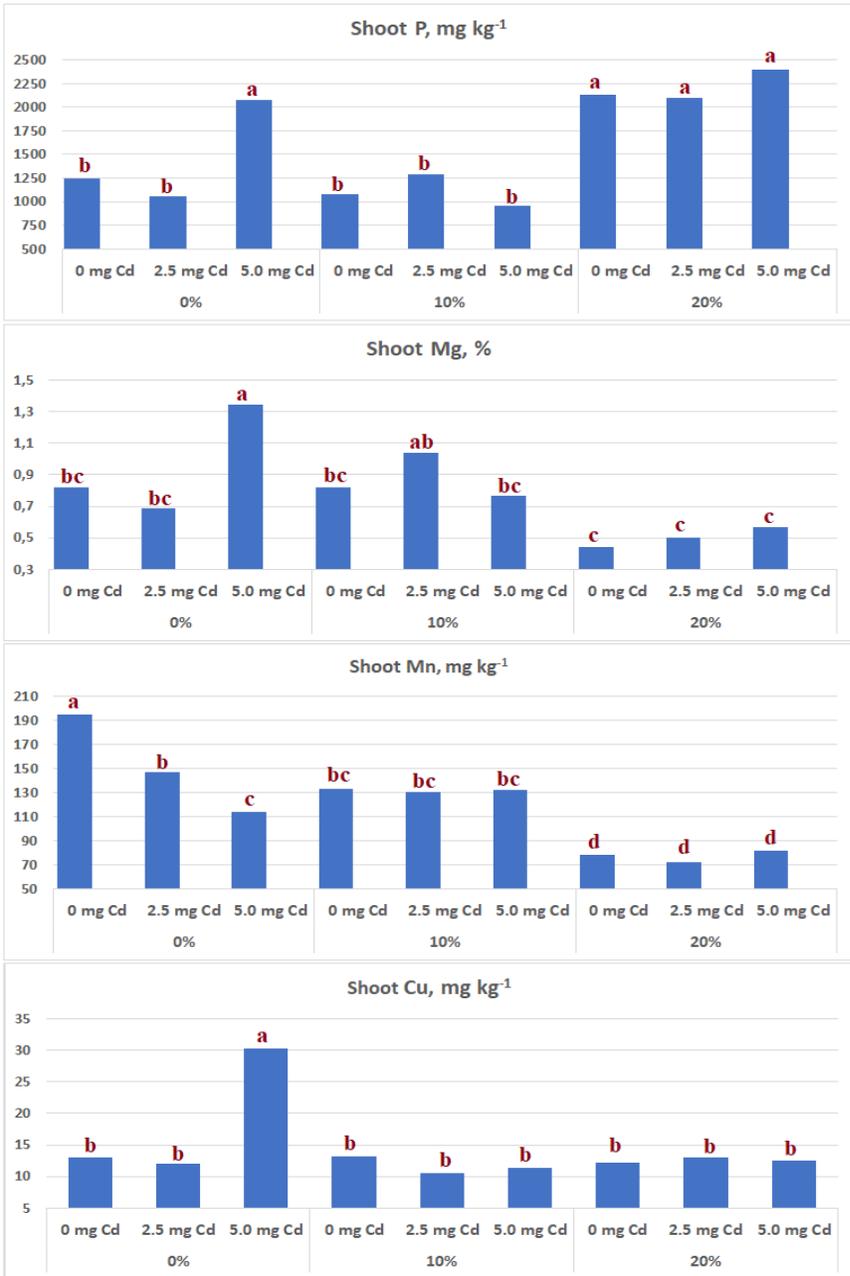
Mean values with different lower-case letters among columns are significantly different at p< 0.05

Effects of peat and cadmium applications on nutrient contents and comparison of the means according to LSD test are given in Table 6 and 7. Peat applications diminished nutrient contents of roots except Ca and Cu contents. The highest mean Mg (0.0971%), P (3734 mg kg<sup>-1</sup>),

Fe (6183 mg kg<sup>-1</sup>), Mn (458 mg kg<sup>-1</sup>), and Zn (29.79 mg kg<sup>-1</sup>) contents were determined in Pt<sub>0</sub> application.

Fe, Mn, and Zn, contents of roots were decreased by increasing cadmium doses opposite to Cu contents. The lowest Fe (4739 mg kg<sup>-1</sup>), Mn (295 mg kg<sup>-1</sup>) and Zn (16.80 mg kg<sup>-1</sup>) contents were determined in Cd<sub>2</sub> application (Table 7).

Effects of peat and cadmium interaction on the nutrient contents in roots showed differences among the nutrients. The highest means Fe (6665 mg kg<sup>-1</sup>), Mn (542 mg kg<sup>-1</sup>), and Zn (36.55 mg kg<sup>-1</sup>) were obtained Pt<sub>0</sub>Cd<sub>0</sub> application. Although the highest means of Cu content (50.58 mg kg<sup>-1</sup>) was determined in Pt<sub>20</sub>Cd<sub>2</sub> application (Table 7).



**Figure 2:** Effects of PtxCd Interactions on Shoot P, Mg, Mn, and Cu Contents

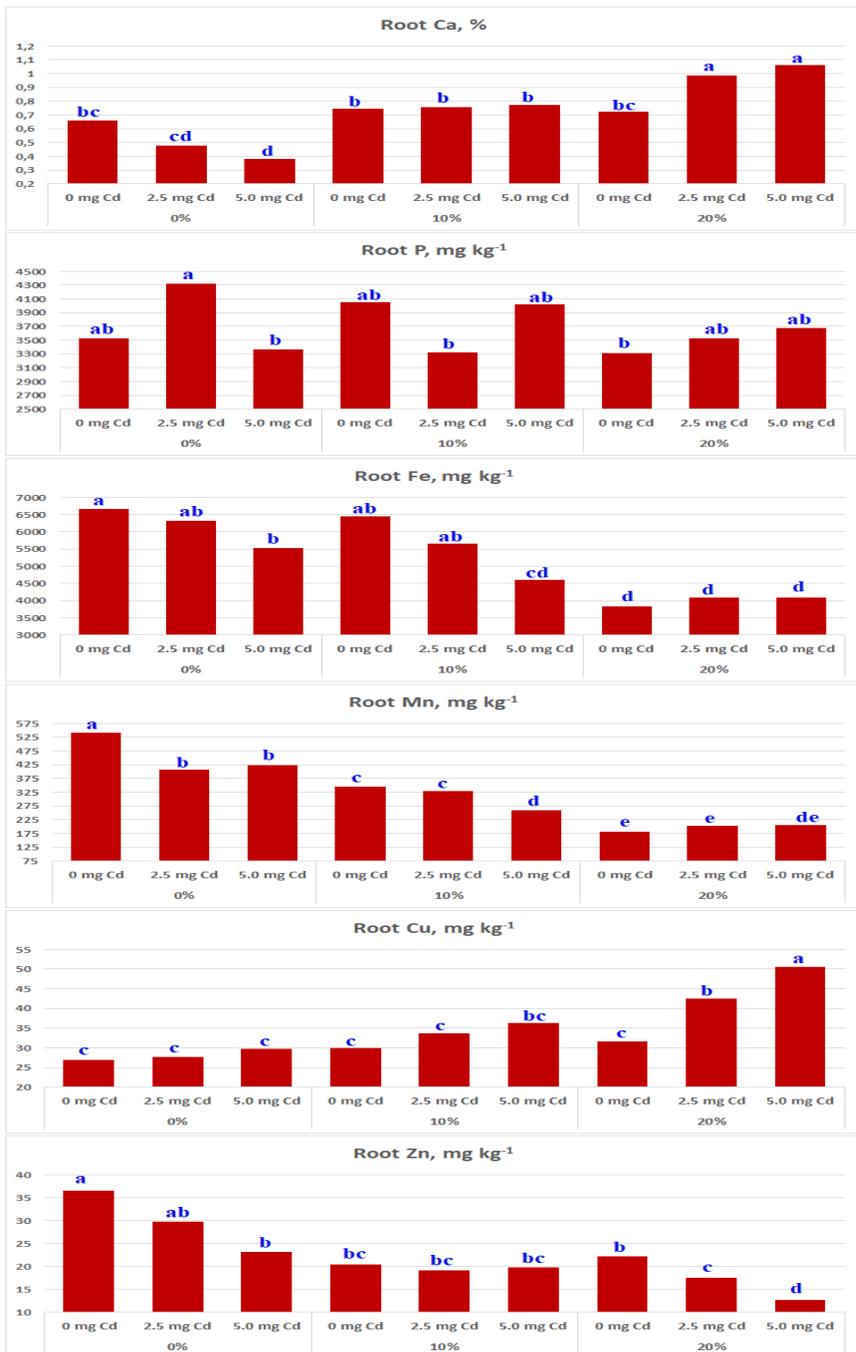


Figure 3: Effects of PtxCd Interactions on Root Ca, P, Fe, Mn, Cu, and Zn Contents

According to the results, it can be concluded that increasing peat concentrations in ratio of soil:peat mixtures had positive effects on seedling criterias on shoot fresh weight, leaf number, shoot length and root fresh weight. Seedling criterias were not influenced by the cadmium application (Table 3). Peat x cadmium interactions was affected root fresh weight and root length (Table 2).

Ameliorative effects of peat on seedling criteria were reported by the most researchers (Gülser et al., 1998; Özman & Ocak, 2002; Çinkılıç, 2008).

Different agricultural wastes improved soil physicochemical properties and increased tomato yield due to their positive effects on some soil quality indexes (Candemir & Gülser, 2011). Demir & Gülser (2015) reported that rice husk compost application as soil organic amendment to the soil in greenhouse generally improved soil quality and tomato yield.

It was also reported that the application of peat had positive effect on grown of willow under heavy metal toxicity conditions (Stanislawska-Glubiak et al., 2012).

The peat treatment to soil contaminated with heavy metals causes changes in the uptake and accumulation of the metals as well as their translocation in plants. These changes lead to higher biomass in maize grown under metal toxicity conditions (Stanislawska-Glubiak et al., 2012).

According to the results, Ca, P, Fe and Zn contents in shoots were increased by 20% peat application. It can be thought that increases in these nutrients were caused by the ameliorative effects of peat such as supplying optimum air-water condition and nutrient contents to soil. Cd<sub>1</sub> and Cd<sub>2</sub> applications decreased Fe, Zn, Cu and Mn contents of shoots opposite to P compared with Cd<sub>0</sub> application (Table 6, Table 7). While the highest means P (1809 mg kg<sup>-1</sup>), were obtained in Cd<sub>2</sub> application, the highest mean Fe (246 mg kg<sup>-1</sup>), Mn (135 mg kg<sup>-1</sup>), Zn (6.57 mg kg<sup>-1</sup>) and Cu (18.47 mg kg<sup>-1</sup>) were in Cd<sub>0</sub> application (Table 6, Table 7). The decreases in Mn and Zn and increases in Fe and Cu contents of shoots were caused by cadmium treatments which were reported by the most researchers (Bowler et al., 1992; Yang et al., 1996; Hernandez et al., 1998; Hagemeyer, 1999; Liu et al., 2001; Wang et al., 2007). Increasing cadmium doses also increased Cu contents in roots. The highest means Cu contents of roots were obtained Cd<sub>1</sub> and Cd<sub>2</sub> doses as 34.64 mg kg<sup>-1</sup> and 38.88 mg kg<sup>-1</sup>, respectively. The increases of Cu contents in roots were like the results reported by Liu et al. (2001). Fe and Cu contents of shoots were lower in peat applications than in no peat application. It was possible that peat inhibited Cd accumulation, Fe and Cu uptake by the seedlings. When peat and cadmium interactions were considered, the highest means of phosphorus content in shoots was obtained in Pt<sub>20</sub>Cd<sub>2</sub> application as 2399 mg kg<sup>-1</sup> respectively (Figure 2). The highest means of copper contents in shoots were determined in Pt<sub>0</sub>Cd<sub>2</sub> application as 30.34 mg kg<sup>-1</sup> (Figure 3).

There are numerous researches on changes of Fe concentrations in the presence of Cd. They were reported that iron concentrations in lettuce (Gárate et al., 1993), tomato (Moral et al., 1994), maize (Hernández et al., 1996) and bean (Chaouiet al., 1997) were not well correlated with levels of Cd applied.

The researchers and agricultural sciences focused Ensuring the sustainable production of healthy food and eco-friendly products due to high demands by consumers in various countries in the world (Willer & Lernoud, 2018).

It is thought that the results obtained from this research could be useful to ameliorate soils expose heavy metal contamination with peat applications as organic soil conditioner. On the other hand, peat applications will be increase plant growth under heavy metal contaminated soils.

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## **CHAPTER XII**

### **PHYTOREMEDIATION OF ORGANIC POLLUTANTS USING TRANSGENIC TOBACCO**

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## 1. INTRODUCTION

Organic pollutants have a very large group of compounds such as polycyclic aromatic hydrocarbons (PAHs), organophosphate insecticides (diazinon, parathion), chlorinated pesticides (PCP, 2,4-D), chlorinated compounds like polychlorinated biphenyls (PCBs), explosives [2,4,6,-trinitrotoluene (TNT), dinitrotoluene (DNT)], surfactants (detergents), and total petroleum hydrocarbons (Macek et al., 2000; Kösesakal et al., 2015; Zhang et al., 2017a; Tanwir et al., 2021). The Agency for Toxic Substances and Disease Registry of the United States (USATSDR) reported 275 toxic substances, which are dangerous for human health when they are incorporated into the food chain (USATSDR, 2019). The first 10 of most toxic and dangerous organic (Vinyl Chloride, PCBs, Benzene, Benzo (A) Pyrene, PAHs and Benzo (B) Fluoranthene) and inorganic (Arsenic (As), lead (Pb) and mercury (Hg), cadmium (Cd)) pollutants for human health and other living organism are given in Table 1.

Unlike inorganic pollutants, organic pollutants can degrade and convert into other compounds. Some of these compounds may remain in the environment for long years and cause harmful effects (Tanwir et al., 2021). Especially, persistent organic pollutants (POPs) are extremely resistant to chemical and biological degradation; they do not clear away and are easily moved or transported from the air, water, soil and they accumulate in the food chain (Istanbulluoglu & Tekbas, 2013).

**Table 1:** The Agency for Toxic Substances and Disease Registry Substance Priority List (USATSDR, 2019)

2019 Rank	Substance Name	Total Points	CAS RN*
1	Arsenic (As)	1676	7440-38-2
2	Lead (Pb)	1531	7439-92-1
3	Mercury (Hg)	1458	7439-97-6
4	Vinyl Chloride	1356	75-01-4
5	Polychlorinated Biphenyls (PCBs)	1345	1336-36-3
6	Benzene	1327	71-43-2
7	Cadmium (Cd)	1318	7440-43-9
8	Benzo (A) Pyrene	1307	50-32-8
9	Polycyclic Aromatic Hydrocarbons (PAHs)	1278	130498-29-2
10	Benzo (B) Fluoranthene	1253	205-99-2

\*CAS RN = Chemical Abstracts Service Registry Number

Due to the unpredictable effects of these organic pollutants on the environment, many countries have agreed to reduce or eliminate the production, use, and/or release of some of them under the Stockholm Convention.

The most important sources of organic pollutants are industrial and agricultural products. Antibiotics, herbicides, polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (PCDD/Fs), PCBs, etc. are organic pollutants, which are frequently used in agricultural activities. These harmful pollutants are set free into the environment because of their use in protection of crops from insects, pests, weeds,

diseases and other farm management activities; and are usually released as waste-water (Kösesakal et al., 2015; Zhang et al., 2017a; Tanwir et al., 2021).

The improvement of remediation methods to clean up these hazardous organic pollutants from contaminated areas is of great importance for the inhibition of their harmful effects on living organisms. There are different methods, such as chemical, biological, and physical methods, used for the reclamation of contaminated areas. However, physical and chemical methods are quite expensive and difficult to apply. Therefore, phytoremediation, which is an effective, cheap, publicly accepted, and aesthetically pleasing method, is preferred for cleaning pollutants from contaminated areas (Abhilash et al., 2009; Torbati, 2015; Kösesakal et al., 2015; Mousavi Kouhi et al., 2016; Sarwar et al., 2017). Phytoremediation means using accumulator plants to extract and remove organic, and inorganic pollutants from the environment (soil, air and water). This remediation technique is primarily used for the cleaning of inorganic heavy metals from contaminated areas. However, recent studies have shown that it can also be used for the remediation of organic contaminants such as pesticides, explosives, Polycyclic Aromatic Hydrocarbons (PAHs) and chlorinated solvents. Furthermore, the process of removing toxic organic substances by plants is generally very slow, which leads to the accumulation of toxic substances in the tissues of plants. Afterwards, these toxic substances could be released into the surroundings and they have to be rendered harmless for living organisms (Abhilash et al., 2009). For the effective phytoremediation

process, the plants have to have some important properties such as deep rooting, high biomass, fast-growing, and high tolerance to contaminants.

There are many plants used in the phytoremediation method. One of the ideal plants for the phytoremediation method is tobacco (*Nicotiana tabacum*), which has a fast growth, high biomass production, deep rooting, and high pollutant accumulation. The development of genetic engineering has led the generation of more effective transgenic plants for decontamination of organic pollutants. The organic pollutant accumulation and detoxification capacity of the tobacco plants can be enhanced by transferring a new gene(s) from different organisms.

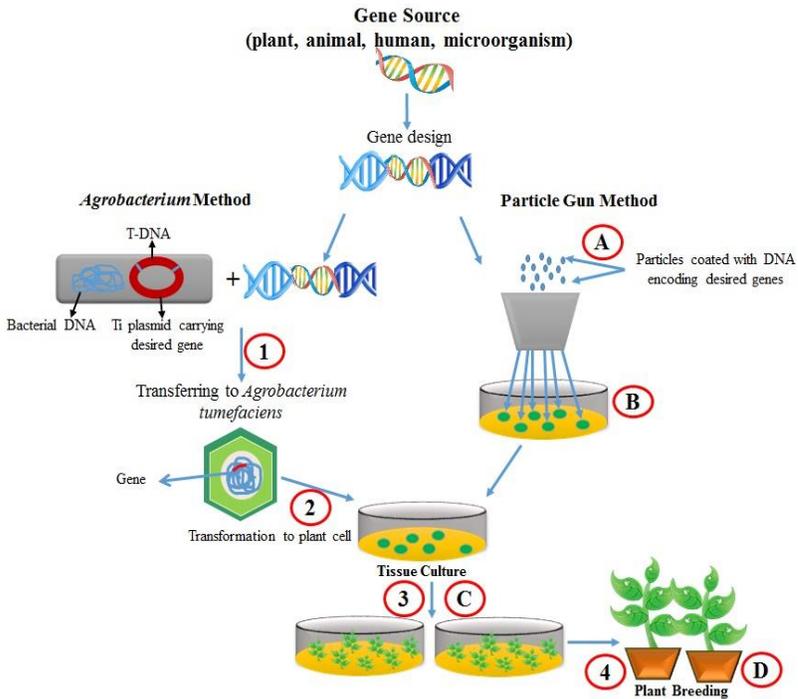
Tobacco is the first genetically modified plant, which is still described as "Cinderella of Plant Biotechnology" in genetic engineering (Ganapathi et al., 2004; Rajeev Kumar, 2015). It is a model plant for genetic engineering. In addition, some properties (high biomass production, fast-growing, producing thousands of seeds in a single capsule, etc.) of tobacco are also suitable for genetic engineering. Tobacco can transfer its genetic characteristics to the next generation by producing seeds (approximately within 3 months) (Evangelou et al., 2007; Ganapati et al., 2004; Jube & Borthakur, 2007; Rajeev Kumar, 2015). However, the main risk of using transgenic tobacco plants to clean up organic contaminants is the gene escape from transgenic tobacco to wild types. Tobacco is a self-pollinating plant. However, its pollen can be carried along with the wind to the wild plants during the flowering stage. Thus, transgenic tobacco plants should be harvested

before flowering to prevent the uncontrolled spread of pollens and seeds (Evangelou et al., 2007; Kotrba et al., 2009).

Transgenic tobacco plants generated for phytoremediation of organic contaminants, some studies of transgenic tobacco plants used for phytoremediation of organic pollutants, uptake mechanism of transgenic tobacco plants, and future of using transgenic tobacco for phytoremediation studies were discussed in this review.

## **2. GENERATION OF TRANSGENIC TOBACCO**

The genes from different organisms were isolated and inserted into tobacco for phytoremediation of organic contaminants. The first step of generating transgenic tobacco is DNA extraction from the desired organism (plant, microorganism, human or animal). After the modification, the desired gene(s) could be transferred with one or a few traits into plants with different methods such as *Agrobacterium*, biolistics (gene gun), electroporation. The most popular and effective transformation processes are *Agrobacterium tumefaciens* and particle bombardment (biolistic or gene gun) transformations, which are schematically presented in Figure 1 (Daghan, 2019). The main principle of these methods is to transport the desired gene(s) into the nucleus of a plant cell. Transformed plant cells are then regenerated into transgenic plants. The transgenic plants are grown till the produced seed stage in greenhouses (Narusaka et al., 2012; Zhang et al., 2016).



**Figure 1:** The processes of transformation and generations of the transgenic tobacco plant by Agrobacterium-mediated and particle bombardment methods. The Agrobacterium-mediated transfer method involves the following steps; (1) transfer of the plasmid carrying desire gene T-DNA into the *Agrobacterium tumefaciens*. (2) The transformation of *Agrobacterium* containing T-DNA into the plant cell. (3) The integration of T-DNA into the plant genome. (4) Plant breeding. The transformation process of particle bombardment includes the following steps; (A) The DNA encoding desired genes coated with particle bombardment methods. (B) The injection of DNA-coated particles into the plant using a particle gun. (C) Regeneration into the whole plant in tissue culture. (D) Plant breeding in the greenhouse (Daghan, 2019)

### 3. PHYTOREMEDIATION OF ORGANIC POLLUTANTS BY TRANSGENIC TOBACCO

Many researchers have done a research on the intake, accumulation, and toxicity of organic pollutants by the plant. Transgenic plants over-expressing different gene sources were introduced into the tobacco for investigation of their phytoremediation capacities for herbicides,

explosives, PCBs, etc. (Dhankher et al., 2012; Feng et al., 2017). According to Feng et al. (2017), the first transgenic plants with the enhanced metabolism of organic pollutants targeted explosives (French et al., 1999) and halogenated compounds (Doty et al., 2000) in tobacco plants. Some examples of transgenic tobacco plants used for phytoremediation of organic pollutants are given in Table 2.

**Table 2:** Different Genes and Their Effects of Transgenic Tobacco Plants Developed For Phytoremediation of Some Organic Pollutants

Organic Pollutant	Transferred Gene	Gene Source	References
Anthracene	<i>Trichoderma virens</i> (TvGST)	<i>Trichoderma virens</i>	Dixit et al., (2011)
PCBs mixture (Delor 103)	bphC gene	Bacterial	Viktorova et al., (2014)
Insecticide	CYP2E1	Human cytochrome	Singh et al., (2011)
TNT	<i>Nitroreductase</i> gene (nfsI)	Bacterial	Zhang et al., (2017b)
			Travis et al., (2007)
TCE	hCYP2E1	Mammalian cytochrome P450	Doty et al., (2000)
Benzene	hCYP2E1	Mammalian cytochrome P450	James et al., (2008)
Toluene			

Pesticides are used intensively for the protection of crops from insects, pathogens, and weeds by farmers. They are also very often used for the prevention of spoilage of harvested and stored crops (Dhankher et al.,

2012; Tanwir et al., 2021). The pesticides could accumulate in the soil with plants application and residues of pesticides could enter into food again via the plant uptake because of their long-term persistence and mobility. Because of that, pesticides are becoming toxic for living organisms. In phytoremediation, transgenic plants expressing specific pesticide-removing enzymes have been developed over the last decade. When pesticides are reduced to non-toxic metabolites or fully mineralized by certain transgenic plants, the plants can be safely disposed of (Gül & Yavuz, 2018). Some studies of using transgenic tobacco plants for phytoremediation of pesticide are given below.

*Trichoderma virens* (TvGST) gene was cloned into tobacco via *Agrobacterium* transformation for the study of tolerance, remediation and the degradation of anthracene, which is a recalcitrant poly aromatic hydrocarbon. The T0 plants were tested in hydroponic and soil experiments and the T1 plants were tested *in vitro* conditions with exposure to <sup>14</sup>C anthracene. All experiments results showed that the fungal GST gene enhanced tolerance to anthracene in transgenic plants. The transgenic plant uptake was 1.5 times more <sup>14</sup>C anthracene in hydroponic and 1.3 times higher <sup>14</sup>C anthracene in the soil as compared to wild-type plants. On the other hand, T0 and T1 transgenic plants were degraded anthracene to naphthalene derivatives in hydroponic and *in vitro* experiments. This may indicate that transgenic plants could degrade anthracene to naphthalene derivatives. Because of the over-expression of TvGST gene in tobacco, this could be the enhancing tolerance to the anthracene (Dixit et al., 2011)

Transgenic tobacco was generated with bacterial *bphC* gene fusion with different markers ( $\beta$ -glucuronidase (GUS), luciferase (LUC) and histidine (His) used for degradation pathway of polychlorinated biphenyls (PCBs). The transgenic tobacco lines decreased the higher total PCB content from the real contaminated soil than wild-type tobacco. The transgenic tobacco plants showed higher tolerance to the commercial PCBs compared to the control plant (Viktorova et al., 2014).

Singh et al. (2011) tested the potential of transgenic tobacco plants expressing a human cytochrome CYP2E1 gene and the capability for phytoremediation of persistent organo-chlorine insecticide Lindane ( $\gamma$ -hexachlorocyclohexane), in hydroponic and soil experiments. Transgenic tobacco showed higher tolerance to Lindane than wild-type tobacco in both test mediums. The gene (CYP2E1) enhanced tolerance to the Lindane in tobacco plants.

Zhang et al. (2017b) reported that phytoremediation of TNT explosives by transgenic tobacco plants which have bacterial *nfsI* gene. The bacterial nitroreductase gene (*nfsI*) was transformed into tobacco by particle bombardment. The T1 transgenic tobacco seeds germinated in MS medium contain 0-20 and 40  $\mu$ M TNT for 14. After that, 20 of the seedlings were tested in liquid MS medium with 0-20-30-40  $\mu$ M TNT for 48 hours. Both experiment results showed that the transgenic tobacco expressing *nfsI* accumulated high-level TNT with high biomass production compared to the wild-type tobacco plant (Zhang et al., 2017b).

Another successful experiment was conducted by Travis et al., (2007), with a bacterial nitroreductase gene transferred the tobacco plants detoxified soil polluted with the TNT (2,4,6-trinitrotoluene). The results showed that the microbial population and metabolic activity significantly increased in the rhizosphere of transgenic tobacco compared to wild-type tobacco.

Trichloroethylene (TCE) is one of the most common organic solvents, which cause environmental pollution. The remediation of TCE by using air sparging, potassium permanganate, anaerobic biodegradation etc. methods are expensive, and not easy to apply. Another important subject is that these remediation methods can cause another environmental pollution problem. Using genetically modified plants to remediate organic solvent TCE contaminated areas is a more effective method (Dhankher et al., 2012).

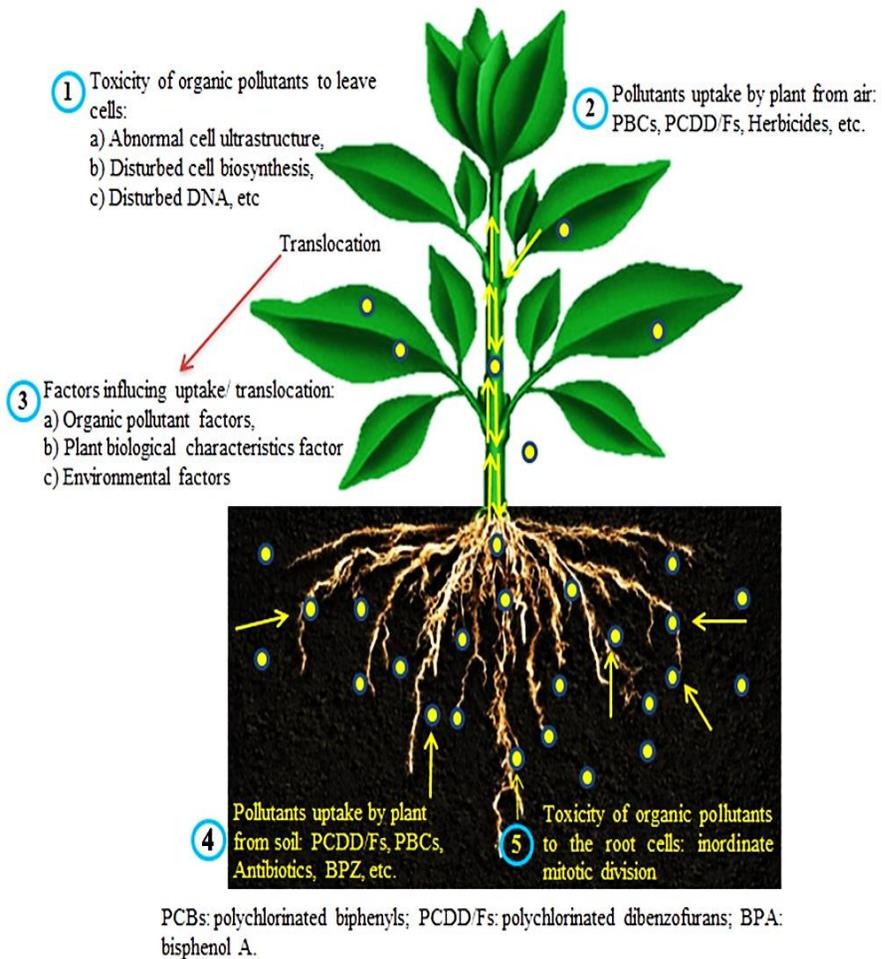
Doty et al. (2000) generated the transgenic tobacco plant that contained mammalian cytochrome P450 enzyme gene (hCYP2E1) to increment the phytoremediation potential of trichloroethylene (TCE). They reported that over-expression of the hCYP2E1 gene in tobacco plants substantially increased the metabolism of TCE. The transgenic plants were able to increase the amount of TCE up to 640 times compared with control plants (Doty et al., 2000). The same gene (single human cytochrome P450, 2E1) was introduced into tobacco (*Nicotiana tabacum* cv. Xanthii) by James et al., (2008). The transgenic tobacco plants removed more toluene and benzene compared to the control plants.

Further studies related to the investigation of improving transgenic tobacco plants for phytoremediation of different kind of organic pollutants are still required.

#### **4. MECHANISMS OF ORGANIC POLLUTANT UPTAKE AND ACCUMULATION OF TOBACCO**

The development and growth of plants depend upon genetic and environmental factors. Especially chemicals are important environmental factors that produce harmful effects on plants. Organic pollutants (herbicides, polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDD), etc.) are released into the environment because of anthropogenic activities.

Plants can uptake some organic pollutants such as PCBs, PCDD/Fs, herbicides, etc. from the air by their leaves and some of them such as PCBs, PCDD/Fs, Antibiotics, BPA, etc. from the soil by roots as shown in Figure 2 (Zhang et al., 2017a; Macek et al., 2000). Organic pollutant types, properties, and concentrations are affecting their uptake and accumulation by plants. On the other hand, soil properties (pH, soil organic matter, texture, etc.), genetic characteristics of plants, and pollution accumulation capacities of plants are affecting phytoremediation of organic pollutants.



**Figure 2:** The Effects of Uptake and Translocation Mechanism of Organic Pollutants by Plants (adapted by Zhang et al., 2017a)

Organic pollutants can enter from the soil solution to the roots of the plant by a passive or active uptake mechanism. After the uptake of organic pollutants by roots, they are transported with xylem (from root to leaves by water) and phloem (from leaves to roots) tubes to other parts of the plants. Both transportations are the small size of organic

pollutants that have higher permeability in membranes than big molecule size of organics. For this reason, they can be easily transported in phloem and xylem. These harmful pollutants cause damage to the tissues of plants. They can disturb DNA, cell biosynthesis and cause abnormal cell ultrastructure in the leaf cells. Toxicity of organic pollutants in the roots can cause inordinate mitotic divisions and damages in the root cells (Zhang et al., 2017a).

## **CONCLUSION**

The genetic engineering of plants is a relatively new and evolving field facilitating the remediation of soils contaminated with organic pollutants. Genetic engineering will make it feasible and manage the capacity of tobacco plants to detoxify, tolerate, and accumulate organic contaminants and thus create an ideal tobacco plant to clean up organic contaminants in the future. Biotechnology has already been successfully employed to increase the tolerance properties of tobacco plants, and to enhance organic contaminant's detoxification. More studies are needed in order to improve transgenic tobacco plants for phytoremediation of soils contaminated with different kind of organic pollutants. Nevertheless, the monitoring of organic pollutant movements and accumulation in the soil, air, water, and plants are important because of their toxic effects on living organisms.

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## **CHAPTER XIII**

### **BIOPESTICIDES IN PLANT PROTECTION**

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## **INTRODUCTION**

Recently, as an alternative to synthetic pesticides, interest in more environmentally friendly natural products with pesticide action has been growing. In addition to the importance of their use in organic agricultural production, one of the goals is their application in integrated pest management (IPM), as well as in conventional agriculture.

Biopesticides are plant protection products (PPPs) derived from such natural materials as animals, plants, bacteria and certain minerals (US EPA, 2021), and products synthesized by organisms. The benefits of biopesticides are numerous - natural origin; less toxicity; impact only on the target group of pests; shorter pre-harvest interval; application in a wide range crops. These compounds rapidly degraded in the environment, and the result is less exposure of the plant and less side effects on the environment, and thus contribution to the preservation of biodiversity, since biopesticides are more selective than chemicals.

In addition, the use of biological agents in integrated pest management enables the development of sustainable agricultural production and reduces the use of chemicals. Their mode of action is usually different from conventional PPPs, which is helpful in the control of resistant populations of harmful organisms, as well as in anti-resistance strategies and managing the sensitivity of controlled species to chemicals (Cawoy et al., 2011).

However, biopesticides have certain disadvantages. They are more difficult to register for market use, have a narrower spectrum of action, act more slowly than chemicals, act preventively (biofungicides), have a shorter expiration date, more expensive, and may be incompatible with other compounds and lowering harm thresholds.

It is especially important to emphasize the importance of biopesticides in organic agriculture. Plant protection in organic production is not a simple replacement of plant protection products used in conventional agriculture with those allowed in organic production (Milenković, 2007). The primary choice in the control of harmful organisms in organic agriculture must be preventive measures – appropriate treatment of plant remains and appropriate hygiene measures, cultivation of resistant varieties, healthy reproductive material, crop rotation, beneficial organisms, mixing plant species, buffer zones, appropriate sowing time, selection plots, and other agrotechnical, mechanical and biological measures.

Besides biopesticides, plant protection in organic agriculture production includes other measures such as healthy plant material, appropriate resistant varieties, crop rotation, time of sowing/planting, and abiotic factors (Močević & Šunjka, 2019). All of the above is applied in order to maintain the level of harmful organisms below the threshold of economic harmfulness, and only if there is a justified reason and necessity, biopesticides or chemical pesticides allowed for use in organic agricultural products are applied.

The aim of this study is to provide an overview of the importance of biopesticides, their classification, benefits and disadvantages, as well as the results of their application, in order to increase commercial use.

## **1. TYPES OF BIOPESTICIDES**

Biopesticides could be classified into three groups:

- microbial pesticides,
- biochemical pesticides and
- Plant-Incorporated-Protectants (PIPs).

### **1.1. Microbial Pesticides**

Microbial pesticides contain selected species of fungi, bacteria, viruses or protozoa. These beneficial organisms produce vitamins, enzymes and hormones that can influence the immune systems of plants, increasing their resistance or competing with harmful organisms. Nowadays, there is a significant interest in the application of the organisms, such as bacteria, fungi and viruses, in the control of pathogens, pests and weeds. They represent about 30% of total biopesticides production and sale.

The specific mode of action of microbial pesticides is based on the fight for space and food, direct antagonism in relation to the growth of the target organism and immunization of the host plant (Grahovac, 2014). In the formulated product, microorganisms have to have a strong competition with the pest population, a high degree of ability to survive and adapt to the new conditions and to achieve the best efficiency.

## **1.2. Biochemical Pesticides**

Biochemical pesticides are substances of natural origin used for the control of harmful organisms. They are composed of substances derived from plants, animals, minerals, insects, etc. Also, they are products of the microorganisms metabolism and plants (toxins, crystals, spores and antibiotics), which protect cultivated plants from pests, while being harmless to humans and environmentally safe. The most important biochemical pesticides are botanical pesticides, i.e. plant extracts and essential oils. For a long time, it has been known that plants have different mechanisms for the protection from various pests, and the use of plant extracts in the control of harmful organisms is considered as old as plant protection (Zibae, 2011).

Therefore, numerous studies investigate the potential pesticide activity of various substances isolated from plants. According to Prakash & Rao (1997), 866 different plant species contain substances with pesticide activity. Botanical pesticides are environmentally friendly due to their fast degradation and the absence of negative influence on non-target organisms.

### **1.2.1 Plant Extracts**

Plant extracts are the oldest and simplest plant-based products. Extracts of over a million plants species are used worldwide for various purposes (medical, food, agricultural). Plant extracts are characterized by simplicity in the preparation and low cost. Nowadays, there is a growing interest in their use in plant protection, as an alternative to synthetic

pesticides, and often as a part of IPM. In 400 BC, baby lice were removed using powder obtained from pyrethrum (*Tanacetum cinerariifolium*), while the first botanical insecticide was applied in the 17<sup>th</sup> century in the form of crushed tobacco leaves against plum harmful beetles. From ancient times, it was considered that gladiolus (*Tropaeolum majus*) sown next to the fruit tree and wrapped around it greatly reduces the presence of plant aphids, as well as nettles (*Urtica dioica*).

The advantages of the use of plant extracts in agriculture are numerous - they quickly metabolize, thus reducing the risk of residues, and due to the presence of numerous biologically active substances contained in one extract, it is difficult for harmful organisms to develop resistance. Many extracts are also selective for non-target and beneficial organisms and safe for humans, animals and the environment.

The pesticide activity of many compounds has been discovered, thus they can also be synthesized as chemical analogues. This is of great importance for the agrochemical industry, especially if it is a new biologically active substance.

### **1.2.2 Essential oils**

Essential oils (EO) are a complex mixture of hydrocarbons, alcohols, ketones, aldehydes, acids, esters and other aliphatic, acyclic, heterocyclic compounds. They are liquids extracted from plants, containing a specific combination of active ingredients, whose amount, presence and specificity depend on the soil type, geographical region,

climate, altitude, harvesting, storage and production (Miletić et al., 2013). EO are plant products, synthesized and collected in structures on the surface (secretory trichomes) or inside (secretory cells, cavities, channels) of the plant (Figure 1).



**Figure 1:** Essential Oil-Secretory Cells of Giger (*Zingiber officinale*) and Mint (*Mentha piperita*) (URL-1)

The highest concentrations of EO are in the leaf, root, flower and fruit. However, they are presented in a small amount (about 1%). As secondary metabolites, essential oils are important for plant life; they attract pollinators, protect plants and repel harmful species.

In the environment, they are unstable, since natural antioxidants degrade very fast after the extraction. Therefore, they have to be stored in a dark, in glass containers. Mostly, they are colorless or poorly colored, volatile, clear. Some essential oils have specific colors (e.g. cinnamon and clove essential oils are brownish red). Essential oils are lipophilic, concentrated and soluble in non-polar organic solvents (petroleum ether, ether, hexane, xylene, methylene chloride, toluene), ethanol and fatty oils, while they are not dissolved in water. It is

possible to obtain aqueous solutions of water-soluble constituents of essential oils, which are obtained e.g. as by-products, during the extraction by steam distillation or by shaking the essential oils with water. Aqueous solutions obtained in this way are aromatic waters.

The scent of essential oils comes from ingredients with oxygen functional groups. It is intense even if the ingredients are presented in low concentrations (e.g. lemon essential oil).

There are about 17,500 aromatic species that mostly belong to families *Myrtaceae*, *Lauraceae*, *Rutaceae*, *Lamiaceae*, *Asteraceae*, *Apiaceae*, *Cupressaceae*, *Poaceae*, *Zingiberaceae*, and *Piperaceae*. Species belonging to the fam. *Lamiaceae*, *Asteraceae*, *Lauraceae*, *Zingiberaceae*, *Myrtaceae*, *Rutaceae*, *Apiaceae*, *Pinaceae* are the richest in essential oils.

Essential oils accumulate in vegetative and reproductive organs such as flower (bergamot and tuberose tree), leaves (citronella, eucalyptus), bark (cinnamon), tree (rosewood, sandalwood), roots (vetiver), rhizomes (turmeric, ginger), fruit (anise, star anise) and seeds (nutmeg). In different parts of the same plant, a similar or completely different composition of essential oil (green and ripe fruit) can be found. Essential oils are formed by the activity of endogenous and exogenous secretory plant tissues. Tissues can appear as individual cells in the parenchyma (*Lauraceae*, *Zingiberaceae*), as cavities of the glandular epithelium (*Rutaceae*, *Myrtaceae*), or as channels (*Apiaceae*). They can sometimes be found in the form of glands and glandular hairs (Duduk

et al., 2010). Processing, purification and other technological procedures yield essential oils, which are not toxic to mammals, birds and fish.

### **1.3. Plant-Incorporated-Protectants (PIPs)**

Plant resistance activators are substances produce from genetic material introduced into the plant. Plants acquire resistance by activating the defense mechanisms.

Transgenic organisms are organisms with the incorporated genetic material of another biological species, responsible for the control of target organisms.

One of the examples is Bt maize containing an insect resistance gene (Coleoptera: Chrysomelidae) isolated from *Bacillus thuringiensis* (Matten et al., 2012; Keweshan et al., 2015). The PIP mechanism of resistance is based on three systems: the presence of morphological barriers, the presence of insect-repellent substances, and toxic substances.

Moreover, biopesticides can be classified based on the active substances, i.e. living organisms (fungi, bacteria, viruses, yeasts, essential oils, plant extracts) and according to biological efficacy, the most important are biofungicides, bioinsecticides and bioherbicides.

## **2. BIOFUNGICIDES**

For the control of phytopathogenic fungi, biofungicides are used. They could be applied in organic, as well as in conventional agricultural production. The use of biofungicides is based on beneficial fungi, bacteria and yeasts, essential oils and plant extracts (Copping, 2009). For the commercial success of biofungicides, the most important is their efficacy in the control of pathogens and the possibility to survive on different plants in different conditions.

There are a few different modes of action of biofungicides:

- direct competition,
- antibiotics,
- predation or parasitism,
- induced host resistance.

Direct competition involves the colonization of the roots of host plants with an organism that is used for biological control (Biological Control Organism, BCO), before infection occurs. In order to compete with the pathogen, the applied organisms must be present in large numbers. The pathogen growth is controlled by the toxins produced by the biological agent (antibiosis). BCO should have antibiotic and antagonistic properties. Predation or parasitism means that an organism used for biological control attacks a pathogenic organism and feeds on it. In this mode of action, the biological agent must be present before the pathogen infection. Furthermore, induced resistance occurs when the

protection system in the plant is activated, not the immune system, but the internal fight to slow down the infection (Grahovac et al., 2009).

Biofungicides are used for seed treatment, for the application on potato before planting or storage, for foliar application, for soaking or spraying seedlings before planting, for irrigation. Biofungicides could be applied in a wide range of plants - field crops, vegetables and fruits, ornamental, medicinal and spice plants.

Mostly, biofungicides are based on microorganisms (fungi and bacteria). Fungi such as *Trichoderma* sp., *Pythium oligandrum*, *Aureobasidium pullulans*, *Fusarium oxysporum*, *Talaromyces flavus* and others most often appear as an active substance of biofungicides. Among bacteria, *Streptomyces griseoviridis*, *Bacillus subtilis*, *Bacillus pumilus*, *Pseudomonas aureofaciens*, *Pseudomonas fluorescens* are most often present in biological products.

In addition to these microbiological fungicides, the most important botanical fungicides are allicin (a volatile substance produced by onion after damage), berberine (an alkaloid isolated from the rhizome of *Berberis aristata*), carvacol or cymophenol, monoterpene phenol present in many essential oils, oregano oil, yellow cedar from Alaska, marjoram (*Origanum majorana*) and thyme (*Thymus vulgaris*), osthol (coumarin component, extracted from dried fruits of *Cnidium monnieri*, and *Angelica pubescens*), sanguinarine (a plant alkaloid, from the group of alohloids, from the group of alohloids) from plants belonging to the

family *Papaveraceae*), santonin (a plant alkaloid isolated from the inflorescence of the plant *Artemisia maritima*).

Fungicidal effect of essential oils isolated from citronella plants (*Cymbopogon winterianus*), cinnamon (*Cinnamomum ceylonicum*), mint (*Mentha piperita* L.), basil (*Ocimum basilicum* L.), rosemary (*Rusmarinus officinalis*), thyme (*Thymus vulgaris*) has been proven. Essential oils of tea tree (*Melaleuca alternifolia* (Maiden and Betche) Cheel) is also well known.

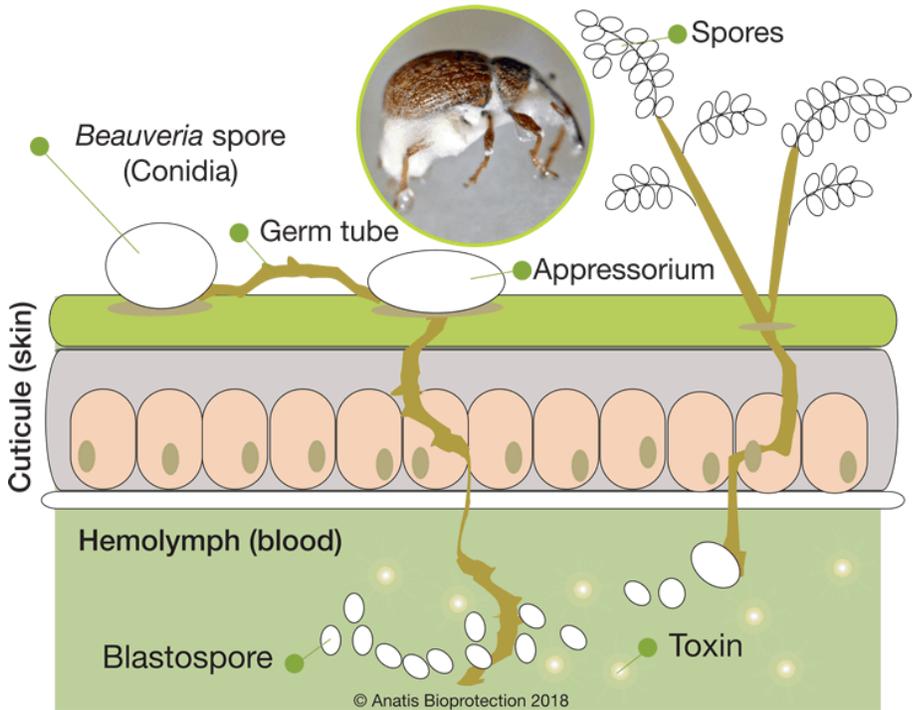
In our previous research antimicrobial activity of several essential oils against strawberry fruit rot pathogens were investigated (Tanovic et al., 2014). Thyme and oregano oils were lethal to *C. acutatum*, *P. obscurans* and to *B. cinerea*. Cinnamon-A showed the best activity against *B. cinerea*, while Chamomile, camphorae, lemon grass and *Helychrysum italicum* essential oils showed the lowest antifungal activity. These results indicate that some of the tested essential oils could be used for the control of investigated pathogens.

It is well known that the apple production is largely affected by the fungi *Colletotrichum gloeosporioides* and *Colletotrichum acutatum*, causal agents of fruit bitter rot. In their control, eco-friendly alternatives to chemical control measures, such as essential oils, became the object of many researches. Grahovac et al. (2012) investigated a fungicide potential of different essential oils. The results indicate that oregano and thyme essential oils have a potential in management of investigated apple pathogens.

Analysis of the efficacy of essential oils of pine and oregano in the control of *Cytospora* sp., in comparison with synthetic fungicide captan, has shown inhibition of mycelial growth in treatment with oregano oil (Iličić et al., 2014).

### **3. BIOINSECTICIDES**

For the control of harmful insects in agriculture production, without side effects to humans, animals and the environment, bioinsecticides are used. The application of natural compounds as insecticides was known in the 17<sup>th</sup> century when the plant extract of nicotine was used in order to control plum blight (*Conotrachelus nenuphar*) and other pests (BPIA, 2017). In 1835. experiments were performed with the application of the fungus *Beauveria bassiana* for the control of some Lepidoptera species (Dara et al., 2018) (Figure 2).



**Figure 2:** Mode of Action *Beauveria bassiana* (URL-2)

The beginning of the 20<sup>th</sup> century brings expansion of research in the field of agriculture and was marked by the use of the bacterium *Bacillus thuringiensis* (Bt) as a bioinsecticide. Nowadays Bt holds the status of the most used insecticide of biological origin (Ibrahim et al., 2010). In the past decade, research and application of bioinsecticides have become increasingly important, due to the intensive development of integrated and organic agricultural production. Bioinsecticides contain a living organism or a natural substance as an active substance (Chandler et al., 2011). Besides direct influence, a biologically active agent can act through metabolic products (toxins, crystals, and antibiotics), or by increasing plant resistance (Copping & Menn, 2000).

Biologically active agents can be fungi, bacteria, viruses, plant extracts and essential oils, parasitoids, as well as actinomycetes, nematodes and others (Chandler et al., 2011; Madduri et al., 2001; Bošković et al., 2018).

The use of azadirachtin as a bioinsecticide is well known in organic farming. It can be also successfully applied in conventional agriculture for the control of different pests (Figure 3) (Močević & Šunjka, 2018).



**Figure 3:** Application of Azadirachtin For Aphids Control In The Organic Artichoke (Original by Močević)

Aphids (fam. Aphididae) are economically very important pests in apple orchards. The most significant is apple aphid (*Aphis pomi* De Geer), which inhabits the leaves and green shoots. Due to feeding, apple aphids cause damages, both direct by feeding with plant juices, and indirect being a vector of plant viruses. Thus insecticide application is necessary in the control of this pest. The aim of the study was to assess the possible use of biological PPP based on azadirachtin, in control of

*A. pomi*, to carry out comparative analysis of the efficacy of biological and chemical insecticides depending on several factors that could significantly be of influence, such as global warming, the possible presence of invasive species of aphids, changes or differences in susceptibility to insecticides. Efficacy of analyzed insecticides was on the same level of significance thus it can be concluded that azadirachtine can ensure adequate protection from *A. pomi* in apple orchards (Vukovic et al., 2015).

Azadirachtin has shown efficacy in the control of *Epicometis hirta* in apple orchards (Vukovic et al., 2019).

Based on previous research it has been confirmed that the plant extracts of *Morus alba* L., *Daucus carota* ssp. *carota* L., *Ambrosia artemisiifolia* L. and *Erigeron canadensis* L. have antifungal, antibacterial and antiviral action. Due to the importance and benefits of bioinsecticide, these plant species represent an important source of bioinsecticides (Tanaskovic et al., 2011).

In our previous research, antifeedint potential of plant extracts of *Ambrosia artemisiifolia* L., *Erigeron Canadensis* L., *Daucus carota* L., *Morus alba* L. and *Aesculus hippocastanum* L. on *Lymantria dispar* L. (Lymantridae) larvae was carried out. The results indicate that plant species, i.e. the origin of extracts, had a significant influence on the feeding intensity of *L. dispar* larvae (Figure 4), while concentration and interaction (plant species x concentration) were not factors of influence (Gvozdenac et al., 2012).



**Figure 4:** Antifeeding Effect, Treatment and Control (Original by Miletić)

Control of storage pests such as *Sitophilus orizae* L., *Tribolium castaneum* Herbst. (Bošković et al., 2020) and *Oryzaephilus surinamensis* L. (Bošković et al., 2021) is very challenging, due to the possibility of the appearance of the residue of conventional pesticides in stored food. Thus, the application of alternative measures is mandatory.

Insecticidal activity of several plant extracts was evaluated for the control of *Sitophilus orizae* L. (Gvozdenac et al., 2013). *S. oryzae* is one of the major economic pests of stored products. Synthetic insecticides are mainly used as grain protectants against storage pest, however, many conventional pesticides can leave residues in food and affect human health, so it is necessary to develop safer means of pest control. Plant extracts of *Erigeron canadensis* and essential oils of *Lavandula angustifolia* and *Ocimum basilicum* are used for the control of storage pest as alternative to chemical insecticides. Their contact, the contact-digestive and repellent effect were evaluated (Vukovic et al., 2016).

Application of essential oils of *Ocimum basilicum* and *Eucalyptus* spp. and ethanol extracts of *Morus alba* and *Ailanthus altissima* on *T. castaneum*, were analyzed (Vukovic et al., 2017). None of the applied essential oils and plant extracts, showed insecticide effect on *T. castaneum*. However, results indicate that essential oil *O. basilicum* showed a repellent activity on *T. castaneum* at higher concentrations.

In our previous study insecticidal activity of three diatomaceous earths (two originated from Serbia and one commercial formulation) on *Rhizopertha dominica* in wheat, barley, rye, oats and triticale grains and their effects on mass of kernels and several properties: adherence, hectolitre mass, moisture, protein and ash contents were evaluated (Perišić et al., 2018).

#### **4. BIOHERBICIDES**

Weed control in organic agricultural production is one of the most challenging issues. For this purpose, only mechanical measures are applied, while chemical control is completely excluded (Vukovic et al., 2013). Currently, bioherbicides are not registered on our market.

In order to reduce the use of synthetic pesticides, in the last twenty years, numerous studies of biological weed control. The very beginning of biological weed control is related to the period after World War II and early 1960s (Carson, 1962), while the earliest experiments included the application of *Fusarium oxysporum* for the control of *Opuntia ficus-indica*. Biological control is based on the use of natural enemies, such as parasitoids, predators, parasites, pathogens or some products of their

metabolism (such as mycotoxins), antagonists or competitors to control the population density of harmful organisms (Van Driesche & Bellows, 1996). According to available, fungi are most often mentioned as bioherbicides, but there are also a large number of plant extracts with bioherbicidal activity based on allelopathy. This implies that bioherbicide application is a sustainable, cheap and environmentally friendly method of control (Charudattan, 2005; Pacanoski, 2015).

Nowadays, the term "allelopathy" means the positive or negative impact of one plant, fungus or microorganism on another. Plant-produced chemicals that cause an allelopathic effect are allelochemicals. Allelochemicals can be found in different parts of plants. Most of them are in the leaves, then in the root, seeds, rhizomes and stems.

Plants with allelopathic activity can be used as bioherbicides, growth regulators, and as a base for the synthesis of new herbicides. The herbicide mesotrione was developed based on allelopathy, even if it is not a complete herbal herbicide. An allelochemical called leptospermum was isolated from the plant *Callistemon citrinus* which was used as a base for mesotrione synthesis, thus allelopathy is an important source of new compounds (Cornes, 2005).

One of the well-known examples is an aqueous extract of the *Sorghum bicolor* and *Helianthus annuus*, which can be used effectively without loss of yield. *Sorghum* is one of the most commonly used species for the analysis of the allelopathic bioherbicidal effect. It shows the

bioherbicidal effect on many types of weeds in different crops such as wheat, cotton and sunflower. The effect of the aqueous solution increases with time and dose, although it is most often used in a concentration of 5% or 10%. They are effective on plants such as *Gossypim hirsutum*, *Glycine max*, *Triticum aestivum* and *Echinochloa crus-galli* (Irshad & Cheema, 2005; Cheema et al., 2012).

Many cultivated plants have allelopathic effects and represent a rich resource for the production and study of bioherbicides. Some rice cultivars show strong allelopathic potential on weeds such as *Echinochloa crus-galli*, *Erodium cicutarium* and *Heteranthera limosa*. Wheat extracts have an effect on some pathogens, pests and weeds such as *Chenopodium album*, *Lolium rigidum* and *Bromus japonica*. Corn and sorghum extract have a pronounced inhibitory effect on germination and development of wild barley. The effect depends on the age of the plant, pH value, nitrogen and carbon content, as well as soil moisture content (Mushtaq et al., 2020). Plants from the family *Asteraceae*, *Convolvulaceae*, *Solanaceae*, *Verbenaceae* are rich in allelochemicals. The essential oil of *Tagetes minutes* inhibits the germination of seeds and seedling growth of weeds *Chenopodium murale*, *Phalaris minor* and *Amaranthus viridis*, by disrupting the chlorophyll content in these weeds (Arora, 2015; Mushtaq et al., 2020). *Ipomoea cairica* leaf extract causes an allelopathic effect on the weeds *Lihularia virgaurea* and *Parthenium hysterophorus*, which makes it a potential herbicide based on natural products (Srivastava et al., 2015).

Some of the oldest known biopesticides do not come from plants or microbes, but from the Earth. Copper, diatomaceous earth, kaolin clay, hydrogen peroxide, potassium bicarbonate, salts and soaps are biopesticides used in some countries, including the United States and Canada. Other products that are potentially classified as biopesticides and come from biological sources, such as by-products from organic processing systems (corn gluten meal, acetic acid) (Bailey et al., 2010), baking soda and canola oil have been used as biopesticides.

## **5. FORMULATIONS**

The formulation of biopesticides aims to ensure the stability of the organism, i.e. the compound that is a part of the plant protection product, during production, distribution and storage, during handling and application. It is necessary to protect the biological agent/compound from the influence of the environment, to increase the activity of the organism during its reproduction, contact or interaction with target organisms. All of this is accomplished by the addition of appropriate non-pesticidal compounds.

Of particular importance for commercial success of formulations is the ability of the microorganism to survive and retain the ability to reproduce under conditions of application, the ability of the compound to retain pesticidal properties, the ability to maintain shelf life and efficiency, market price, ease of handling and application. The formulation of biopesticides needs to be suitable for the existing equipment for the application of PPPs (Boyetchko, 1999), and in most

cases this process takes place in the same way as the production of synthetic pesticides.

The use of essential oils as biopesticides is of great importance, given the established pesticidal activity of essential oils of a number of plant species. However, given their volatility, poor water solubility and oxidizing ability, formulating biopesticides based on essential oils is a major challenge. Today, this can be overcome by introducing appropriate excipients in the formulation process (Ibrahim, 2019).

The development of new carriers of micro and nano sizes has led to the appearance of the methods for micro and nanoencapsulation of active compounds, and polymers (natural or artificial substances consisting of large molecules composed of related series of simple monomers) stand out as the most widely used.

In this way, the compounds are protected from degradation and evaporation, and better stability and efficacy of these types of formulations can be expected (Ibrahim, 2019). On the other hand, it was found that nanoformulations show higher specificity (low toxicity to non-target organisms compared to other commercial formulations), and by increasing the persistence or stability of the active substance at the application site, the amount of biopesticide application decreases. Given their physical state, biopesticide formulations are divided into two basic groups - solid and liquid.

Solid formulations are produced using different technologies, such as spray drying, freeze-drying, or air drying with or without the use of a fluid bed. They are produced by adding binders, dispersants, wetting materials (Tadros, 2005; Knowles, 2008). Each type of formulation is produced in a specific way.

The most important solid formulations:

- dusting powder (DP),
- granules (GR),
- micro granules (MG),
- water-dispersible granules (WG),
- wettable powder (WP).

While biopesticides formulated as DP, GR and MG are directly applied, the application of water-dispersible granules (WG) and wettable powder (WP) implies their dispersion in an appropriate amount of water (Knowles, 2005).

Liquid formulations of biopesticides are produced on the basis of water, oil, polymers or combinations thereof.

Among the most important liquid formulations are:

- concentrated suspension (SC),
- oil dispersion (OD),
- suspo-emulsion (SE),
- capsule suspension (CS),
- emulsion concentrate (EC),

- concentrated solution (SL).

Water-based formulations (SC, SE, CS, SL) require the addition of inert ingredients, such as stabilizers, adhesives, surfactants, paints, antifreeze compounds. On the other hand, formulations containing non-volatile solvents, including oils (such as oil dispersion), help the process of application and retention of the product on the treated object and improve the binding of spores (Gašić & Tanović, 2013).

After the formulation of biopesticides, laboratory and field tests are performed in order to register and place on the market. In this procedure, the efficacy, phytotoxicity and physico-chemical properties of the formulated products are evaluated. Moreover, the concentration of application is determined, and the commercial name is defined. Information on the identity of the active substance, whether it is an organism or a compound, the content of the active substance, the type of formulation, the commercial name, the concentration/amount of application, the organism to be controlled, and the time of application must be on the biopesticide label.

Agricultural production in developed countries is strongly influenced by different factors; some of them are a consequence of consumer demands and some are of ethical importance. This is especially true of plant protection. Global demands for the reduction of the use of chemical pesticides, which are considered harmful to consumers and the environment, affect the development of new, more eco-friendly, sustainable strategies in plant protection. Many pesticides have been excluded from use, due to their potential risk to human health,

environmental pollution, effects on non-target organisms, or the development of resistance of harmful organisms. So, it is necessary to develop new alternative protection systems in the future and they must be implemented as a supplement or replacement to the conventional application of pesticides. Application of biopesticides in combination with other plant protection measures would meet the requirements for the production of safe agricultural products and food, from the aspect of pesticide residues.

## **CONCLUSION**

Agricultural production in developed countries is strongly influenced by different factors; some of them are a consequence of consumer demands and some are of ethical importance. This is especially true of plant protection. Global demands for the reduction of the use of chemical pesticides, which are considered harmful to consumers and the environment, affect the development of new, more eco-friendly, sustainable strategies in plant protection. Many pesticides have been excluded from use, due to their potential risk to human health, environmental pollution, effects on non-target organisms, or the development of resistance of harmful organisms. So, it is necessary to develop new alternative protection systems in the future and they must be implemented as a supplement or replacement to the conventional application of pesticides. Application of biopesticides in combination with other plant protection measures would meet the requirements for the production of safe agricultural products and food, from the aspect of pesticide residues.

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## **CHAPTER XIV**

### **NEW APPROACHES FOR WEED MANAGEMENT**

#### **IN HORTICULTURE**

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## **INTRODUCTION**

From the point of view of government, industry and the media, the year 2050 is a milestone. At this time, the world population is expected to peak at 9 billion people, putting a strain on the global capacity to provide sufficient energy, fresh water and food (Alexandros et al., 2012). Current level of production is insufficient to feed the projected population, these expected needs is seen as a major challenge for humankind. In addition to socio-economic and crop- related problems, there are several biotic and abiotic restrictions on plant production. Weeds are the main abiotic barriers to agricultural production in both developing and industrialized countries. In general, weeds represent the highest potential yield loss for crops, along with pathogens (fungi, bacteria, etc.) and animal (insects, rodents, nematodes, mites, birds, etc.) which are less of a concern (Oerke, 2006). Weeds compete with plants for sunlight, water, nutrients and space. They also harbor insects and pathogens that attack crops. In addition, they destroy native habitats and threaten native plants and animals (Zimdahl, 2018). Weed control is essential to agricultural production and landscape and environmental management and will play an important role in whether we meet the future needs of food production. Researchers have developed and applied new methods with the help of the technology and digitalization in agriculture by means of precision weed management technics with the help of remote sensing, modeling and robotics (Bajva et al., 2015). Because the intensive use of the chemicals in weed control increased rapidly and as a result, undesirable side effects such as herbicide resistance and residue problems that adversely affected human and

environmental health emerged. In order to reduce the negative pressure of chemicals on human health and the environment and at the same time not to reduce the yield of agricultural products. New approaches for weed management generally focus on developing and/or adopting new weed control technologies with the aim of reducing environmental impact. In this paper the potential of the various possible uses of weed control methods are described.

### **1. WEED CONTROL WITH ELECTROMAGNETIC RAYS**

One of new methods of weed control is the use of electromagnetic rays with different wavelengths. These rays are used successfully in many areas such as medicine, space, communication and the food industry. If the necessary improvements will be carried out, the electromagnetic rays, the use of which have been investigated against weeds in recent years, can be effectively used in practice. Particularly in robotic systems that used advanced technologies to differentiate between weeds and crops with the help of sensors, these methods will have the ability to be successfully applied in row crops such as some vegetable. With these systems, the application does not affect the entire area, but only the areas where weeds are present, thereby reducing input and environmental pollution. The amount of research on sensor-based robotic systems that use laser beams to control weeds along with remote sensing techniques has recently increased.

## **1.1. Rays Used in Weed Control**

The rays that are the subject of weed research, physically damage weed seeds and weeds by killing or preventing their development. These rays, known as electromagnetic rays, are formed as a result of periodic events at the atomic level. They are known for their wavelengths and frequencies. Short wave and high-frequency ones carry more energy than long wave and low frequency ones. If these rays hit an obstacle (plant tissue), they are either reflected back, passed through the tissue or absorbed by the tissue. When these rays are absorbed by the plant, the energy contained in the ray passes to the plant and this energy causes some negativities in the plant (Güncan, 2013). Although the researchs on the weed control by means the use of ray are in progress, there are six different types of electromagnetic rays: microwave, UHF, infrared, ultraviolet, gamma rays, and laser.

### **1.1.1. Weed Control with Microwave**

Microwaves are electromagnetic energies of short wavelength (1 mm - 1 m) and high frequency (300 MHz - 3000 MHz) that move at the speed of light (Kitiş & Çavuşoğlu, 2016). The wavelengths that carry high heat energy used in microwave ovens are rays with a frequency of 2 to 4 GHz, called the S band (Zlotorzynski, 1995). Microwave energy has been used in agriculture for soil borne microorganisms and weed seeds (Diprose et al., 1984). Microwaves can be applied both to weed seeds close to the soil surface and to the non-wholly germinated weed seed and weedlings (Şahin, 2014). Advantages of this method is to adsorb rapidly by the plant tissue and microwave radiation is not affect by the

wind and including without residue after application. In the weed control to be carried out post emergence, the microwave emitting system should be focused towards the target. In weeds exposed to these rays, microwaves first pass through the cell wall and are then absorbed by the water molecules in the weed. As a result, the water-containing tissues of the plant are overheated and come out by breaking the cytoplasm cell wall. On the other hand, due to overheating, the proteins in the weeds deteriorate and lose their functions. However, the reaction of every weed species to microwave application is not the same. For example, velvetleaf (*Abutilon theophrasti* Medik.) of the same size and density and broomcorn millet (*Panicum miliaceum* L.) at the same dose of microwave did not control weeds at the same rate. Some studies addressed that monocotyledon weed species are more durable than dicotyledon species, due to the growing point is protected by a sheath (Kitiş & Çavuşoğlu, 2016). For example, 1015 kJ m<sup>2</sup> energy is required to reduce the dry weight of velvetleaf by 90%, while 3433 kJ<sup>2</sup> m energy is required for broomcorn millet (Sartorato et al., 2006). Also Brodie & Holins (2016), indicates that wild radish (*Raphanus raphanistrum* L.) was 100% controlled with 60 J cm<sup>-2</sup> microwave application, 370 J cm<sup>-2</sup> energy was required to completely control annual ryegrass (*Lolium rigidum* Gaudin.). Microwaves can also destroy weed seeds in the soil. When applied over the soil, it can go down to a few cm into the soil and damages the weed seeds in these regions. But sometimes it can promote the germination of seeds of dormant species in the soil. Seed damage by the microwave energy is depend on the combination of the seed moisture content and the energy absorbed per seed (Brodie et al., 2012).

It was also seen that the soil temperature affected the results in microwave applications. When the soil temperature arrives up to the 80 °C whole weed seed germination inhibited (Brodie et al., 2012). Studies not only showed that microwave application was successfully destructed a range of weed seeds in the soil (Davis et al., 1973; Barker & Craker, 1991; Brodie et al., 2009), but also was effective to manage weed plants. For example, Brodie et al. (2007) developed prototype microwave system based on a modified microwave oven for marsh mallow (*Malva parviflora*), fleabane (*Conyza bonariensis*), paddy melon (*Cucumis myriocarpus*) seedlings and plant death occurred after 10 days of application.

In a study a microwave tunnel with conveyor belt designed to test cress, charlock and wild oat seeds. The effect of microwave applied at different exposure duration, weed species and energy density of microwave end up plant death between 4% to 100% (Şahin, 2014). For post emergence weed control, microwave requires less energy consumption because during the soil application for pre-emergence weed control soil water absorb the microwave. It is conceptually similar to the soil fumigation. This method can be useful for post emergence weed control, without disturbing soil for conservation agriculture (Bauer et al., 2020). However, there is need to improve some prototypes to optimise energy consumption.

### **1.1.2. Weed Control with Ultrahigh-Frequency Electromagnetic Fields**

An Ultrahigh-Frequency Electromagnetic Field (UHF), (2450±20 megahertz) is lethal to the various seeds of species and plant comparatively short exposure time (Davis et al., 1971). In some sources, they are included in the lower frequency and higher wavelength portion of microwave rays. UHF waves also have a thermal effect when applied from a strong source, similar to microwaves. Weed control with UHF rays is generally carried out for the control of weed seeds with soil applications. For example, 2450±20 MHz UHF wave was applied to both weed seeds in the soil and germinated plants, and it was observed that an energy of more than 70 J cm<sup>-2</sup> was required to get successful results in the pre-emergence application. In the post-emergence application, it was determined that weeds were more sensitive than monocotyledon weeds. In addition, more success was obtained from wet and heavy textured soils than dry and light textured soils. Similarly, it has been stated that seeds with high moisture content can be controlled more easily or with less energy expenditure than dry seeds or seeds with low moisture content, and even germinated seeds are completely eliminated. UHF waves can be applied against weeds in areas where the crop seeds germinate with delay and are not on the surface.

### 1.1.3. Weed Control with Ultraviolet Rays

Electromagnetic radiation with wavelengths between 100-400 nm and between X-rays and visible light is called ultraviolet (UV). While UV rays are invisible to humans with the eyes, near UV is visible to birds, insects and fish. While some of these rays coming from the sun to the world easily pass through the ozone layer and reach the earth, some of them are retained by the ozone layer. UV-A (320-400 nm) rays are the most common and pose the least danger to our health. The ozone layer allows these rays to pass, that is, to reach the earth. UV-B (290-320 nm) rays are very dangerous rays for living things. The ozone layer traps most of these rays and prevents them from reaching the earth. UV-C (100-290 nm) rays are the most dangerous rays for living things, and the ozone layer traps all these rays and prevents them from reaching the earth.

In various studies, it has been observed that when UV light is applied to plants, almost all the energy is absorbed by the plants and as a result, the plant tissues warm up. In the experiments carried out under greenhouse conditions, UV light at doses ranging from 1 to 100 GJ ha<sup>-1</sup> was detected by shepherd's purse (*Capsella bursa-pastoris* L.) Medik.), common groundsel (*Senecio vulgaris* L.), dwarf nettle (*Urtica urens* L.) and annual bluegrass (*Poa annua* L.) successfully, and that the wet weight of the weed flora can be reduced by about 95%. It has been found that a dose of 10 GJ ha<sup>-1</sup> is required. (Andreasen et al., 1999). As in other radiation applications, factors such as the species of

weed, the stage of development and the height of the UV source above the plant are effective.

Not much research has been done on weed control with UV rays, due to the failure of UV experiments in field conditions, its effect on soil microflora, high energy consumption and the risk of causing mutations in living organisms.

#### **1.1.4. Weed Control with Infrared Rays**

Radiant rays with wavelengths between 750 nm and 1 mm, and frequencies between 300 GHz and 430 THz, larger than visible light and smaller than microwaves. Having many uses such as night vision systems, tracking systems, communication, thermography and heating, the high energy they carry in the form of heat is also used for weed control. Infrared (IR) systems developed for weed control use mostly the energy released by the combustion of petroleum gas derivatives. In such systems, IR energy is produced by heating ceramic or metal surfaces. IR rays were first tested for agricultural purposes in the 1960s as a defoliant in cotton. For the purpose of weed control, studies were carried out by many researchers, especially in Europe, in the 1980s.

A researcher tested four different electrically powered IR emitters on white mustard (*Sinapis alba* L.) and ryegrass (*Lolium italicum*) as test plants. As a result, it was found that weed species and development period are important parameters affecting the result, 200-400 kJ m<sup>-2</sup> energy is needed for these two species, and ryegrass is more durable than white mustard. Today, the use of IR rays alone is very limited due

to the high amount of energy needed to get an effective result in infrared-based systems, the relatively lower heat generation compared to flaming equipment, the slow application speed and the unsuccessful results in some field applications. However, recently, hybrid systems have been developed that combine IR applications with flaming equipment.

### **1.1.5. Laser-Based Weed Control**

After the discovery of laser light, it has been used in various fields such as medicine (Liu et al., 2012), industry (Malinauskas et al., 2016), telecommunications (Adamu et al., 2020) microbiology (Tsuchida et al., 2020) and space science (Wiens et al., 2020) with many advantages. As the usage areas are increased, the development process of the laser is increased impressively and different types have been developed for different usage areas. There are varieties of lasers such as diode laser, CO<sub>2</sub> laser, ion laser and chemical laser. CO<sub>2</sub> lasers are generally used for weed control. These lasers are highly effective and powerful. There are two types of CO<sub>2</sub> lasers: continuous and pulsed. In practice, it is important that the laser source is correctly focused on the target (Kitiş & Çavuşoğlu, 2016). The effect of laser is the contact, that is, it can cause damage only at the point where the beam touches. To cut with laser is performed by targeting the tip meristem, stem or leaves of the weed. The most effective results were obtained when applied to the tip meristem of the weed (Marx et al., 2012). Because the tip meristem is one of the main growth points of the plant. By destroying the most important growth point of the weed, the growth of the weed is

prevented, reduced or completely eliminated. Some studies have been carried out on CO<sub>2</sub> laser beam to cut stems of *Chenopodium album*, *Sinapis arvensis* and the leaves of *Lolium perenne* with three different grown stage and two heights; dry weight of the weeds and laser energy have been analyzed using dose-response model. The result showed that it is crucial to cut plant close from soil surface for avoidance of weed regrown (Heisel et al., 2001). Laser treatment gives acceptable results in the cotyledon stage with the application directed to the apical meristems. The efficacy of laser-based weed control method depends on the wavelength, exposure time, the spot size and the power of the laser. Also effectiveness of laser application depends on the weed species (Mathiassen et al., 2006). A study was carried out to test two lasers and two spot sizes on common chickweed (*Stellaria media* (L.) Vill.), scentless mayweed (*Tripleurospermum inodorum* (L.) Sch.) and oilseed rape (*Brassica napus* L.). The results showed that the application of laser on apical meristems caused significant grown reduction and in some cases killed the weed species (Mathiassen et al., 2006). Another approach for laser-based weed control is laser weeding robot. Xiong et al. (2017), has been developed and tested a prototype laser weeding robot equipped with dual-gimbal laser pointers. The robot was designed to be a mobile platform using a modified small commercial quat bike, a camera to specify the crop and weeds at the cotyledon stages with two steerable gimbals to control the laser pointers. The experiment of robot in indoor environments showed the possibility to irradiate target weeds with lasers and control the platform in real-time realizing continuous weeding. Rakhmatulin & Andreasen

(2020) developed and tested a prototype of relatively cheap laser-based weeding device on couch grass (*Elytrigia repens* (L.) Desv. Ex Nevski) mixed with tomatoes. Three types of lasers (0.3 W, 1 W, 5 W) for identifying weed species and a neural network, for coordinating the laser guidance system were used. Require energy for plant damage depended on plant length. They found that for efficient of exposure time and weed suppress 5 W laser was more convenient than other type of lasers. However, when laser beam became split into two, 5 W type caused damage to the crop. Research showed that it is possible to improve cheap device for laser-based weeding with challenges of crop-weed recognition, exposure time needs to study.

## **2. WEED CONTROL WITH ROBOTS**

In this method, vehicles with remote sensing or sensor technologies, defined as robots, are used. Robots are used in many different fields as well as in agriculture. Robots used in agriculture consist of 3 basic components. These; It consists of sensors that can measure important biological and physical properties in the agricultural system, a system that determines how the information from the sensor system will be used in the agricultural system, and an actuator that activates this system. Artificial vision analysis, GPS, variable rate application techniques and robotic technologies make it possible to control weeds with robots. Thanks to this technology, the required amount of application is made where it is needed. This is also called a variable-level application. There are two types of applications. In the first, the distribution of weeds in the area to be applied is determined with the

help of global positioning devices (GPS). Then, the device moving in the direction of the determined coordinates makes the application. In the second, with the help of a sensor placed on the device, the location of the weeds in the field is instantly detected and the application is made. In both methods, since the application is made only on the weed spots, the number of herbicides or other inputs applied decreases significantly (Çavuşoğlu & Kitiş, 2014).

General purpose autonomous robotic weed control system has four technologies:

- Real-time Kinematics Global Positioning System (RTK GPS) or image acquisition,
- Weed detection and identification (machine vision, hyperspectral imaging, GPS supported),
- Weed control (micro-spray, cutting, thermal, electric) in row crops and
- Mapping (GPS and machine vision).

### **2.1. Artificial Vision Based Robots**

After decades of research and development in different parts of the world, robots have been built for hoe crops that provide a high degree of automation and commercial success. Among these, two types of sensors have had the greatest commercial success: artificial vision and global positioning systems (GPS).

Vision systems require a directional beacon and typically use a forward view of rows of plants. Hough (1962) found and patented one of the

most commonly used machine image methods for identifying plant rows.

## **2.2. Real Time Kinematic GPS Based Robots (RTK-GPS)**

In this system, unobstructed vision should be provided compared to the artificial vision-based system. There should be no obstructions in front of the production area. GPS systems provide an absolute guidance system and, unlike systems with artificial vision, require the crop to be planted using an RTK - GPS guided. RTK GPS systems require a GPS base station to be within approximately 10 km of the RTK GPS-guided tractor or agricultural robot. However, it has the advantage that it is not due to weed density, shady areas, missing plants in rows, or other conditions that degrade the performance of artificial vision systems. Another advantage of this system is that it can be programmed for uneven rows of plants (such as lands with Center Pivot Irrigation System).

For plants planted in rows by preparing the seedbed using commercial RTK GPS automatic tractor systems, the system is said to operate with a precision error of up to 25 mm, usually.

To obtain optimum performance from RTK GPS systems, they should be used in areas with a minimum amount of radio frequency interference, minimal multipath errors (such as reflection of GPS signals near the antenna), and at least four common satellites (to the tractor and base station).

### **2.3. Recognition of Plant Species by Artificial Vision**

The system is used so that plant species can be defined in three general categories. These are biological morphology, spectral features and visual texture. The morphology of plant is based on the leaf's shapes, color, texture and other characteristics (Zhang et al., 2020). The oldest and most comprehensive study on the remote sensing studies are on satellite and aerial images (Moran et al., 1997).

### **2.4. Map Based Robots**

Weed spots in the field are mapped, and applications (thermal, laser, herbicide, etc.) The most widely used global satellite system in map-based applications is GPS (Meyer & Filliat, 2003).

### **2.5. Sensor Based Robots**

In this method, the determination of the parts where weeds are found and the applications (thermal, laser or herbicide) are carried out simultaneously. A sensor-based sensor, which is developed to combat weeds and is usually located at the front of the system, instantly detects the location of the weeds and transmits this information to the application section, which operates on the basis of on/off according to the signals it receives from the computer at the back. In this section, when it receives the signal that weed is present, it applies the input to the desired area, that is, the area where the weed is located, instantly. Working with this system, the robot wanders between the rows of cultivated plants in the field, detects weeds and performs its struggle. This method is divided into two:

### **2.5.1. Reflection Based Optical Sensing Robots**

Objects appear in different colors depending on the reflectance of the light falling on them. In plants, they look different from each other according to their reflectance of light. By means of sensors that detect these different reflections, crop plants and weeds look different. In this way, applications can be made on the detected weeds with this system.

### **2.5.2. Image Processing Robots Using the Camera System**

In this system, plants are defined geometrically, structurally and schematically. Most machine vision for plant species identification is at the leaf geometry level, and some at the whole plant level. In this system, the differences in shape of weed and cultivated plant are defined. With the image transferred to the computer environment, weeds and cultivated plants are separated. Different applications can be made on the detected weeds.

Robots are used in many different fields as well as in agriculture. In the fight against weeds, both map-based and sensor-based different robotic systems have been developed. Sensor-based applications seem to be more advantageous than map-based systems, as they take snapshots and detect weeds in a single step, as well as control applications. Since non-target organisms are not interfered with in the weed control of robots, the amount of pesticide thrown decreases significantly. Studies on this subject in the world are continuing rapidly. Although there are studies on this subject in our country, more research is needed on this subject in order to be applicable and economical in practice.

### 3. THERMAL WEED CONTROL

Thermal weed control plays an important role in weed control in different ecosystems, especially in organic agriculture. This technology is based on the principle of providing thermal energy exchange in above-ground plant parts with high temperature (Ascard et al., 2007). The basis for the development of weed management strategies involving heat or high temperature; It is the death of plants by exposure to high temperatures up to 45-55 °C. High temperatures are lethal for plant tissues as they adversely affect many physiological functions in the cell, such as rupture of the cell membrane, protein denaturation and disruption of enzyme activities. There are many factors that affect heat damage in plants, such as energy input, exposure time, and weed species. Most of the thermal applications require reapplication because it damages the above-ground parts of many plants, and because the underground plant parts reappear after application in perennial plants. In commercial applications today, thermal weed control methods are typically used before the crop emerges, especially if the crop competes poorly with the weeds (Peruzzi et al., 2004; Seaman, 2016), for example in carrot crops with broad-leaved weeds such as *Bidens pilosa* or *Amaranthus* spp. and grass weeds such as *Echinochloa crus-galli*, or in situations in which the culture is more resistant than typical weeds such as corn, (Mutch et al., 2008; Ulloa et al., 2012), for example in post-emergence maize with important weeds such as *Cynodon dactylon* or *Parthenium hysterophorus*. While annual and biennial weeds can be controlled well when exposed to thermal methods, perennial weeds are less affected due to underground rhizomes and roots (Cisneros &

Zandstra, 2008; Stepanovic, 2013). Frequent thermal weed control could therefore lead to monoculture weeds of, for example *Taraxacum* spp. in vineyards. Thermal and chemical weed control methods are generally used at similar developmental stages of weeds.

### **3.1. Flaming**

Flaming is the most effective and most used method among thermal weed control methods by damaging plant tissues with heat. In this method, LPG or propane gas is used as the fuel source (Kitiş, 2010). Propane gas is used in many flamethrowers, but hydrogen as a renewable energy source is considered as an alternative to propane gas. In this method, there are flamethrowers that can be applied manually in small areas, machines that can be integrated into the tractor and applied in large areas. Depending on the characteristics of the application areas, this method can also be risky. For example, its use in weed control is limited in places such as Australia where there is a fire risk.

#### **3.1.1. History**

Flaming is considered as a promising method for weed control in organic farming areas. It is also used in traditional farming. The first device for flaming was developed and used by John A. Craig in 1852 to control weeds in sugarcane.

#### **3.1.2. Mechanism of Action**

Flaming is a method based on the principle of damaging the growth points of weed seedling especially on the soil surface, by applying heat.

The basic principle in this method, unlike burning, is based on the short-term application of high heat, the cell sap in weeds expands and the cell walls explode, and then the plant withers and dies. This method is used in agricultural areas in three ways: pre-sowing, pre-emergence and post-emergence. It has been stated that exacerbation is a successful control method against some weeds and that it can be easily preferred instead of chemical control in some areas.

### **3.1.3. Effect on Growth and Reproduction of Weeds**

According to the studies, while exacerbation is effective in the control of broad-leaved weeds, the control of narrow-leaved weeds has not been found very effective. However, the development period of the weed affects its sensitivity to flame. Effective results were obtained for narrow-leaved weeds from the exacerbation applications made while the weeds were in their early stages. In a study conducted to determine the effects of flaming and hoeing on weed control and sunflower grain yield and yield components in sunflower production, propane gas flaming combined with hoeing was used. As a result, it was determined that the use of flaming and hoeing together in weed control in sunflower increased yield and as a result, it could be used especially in organic agriculture (Tursun et al., 2017).

### **3.1.4. Advantages and Disadvantages**

This method is more advantageous as it does not leave any residue in the plant, soil, water and air compared to chemical use, and does not cause resistance problems in weeds. It also requires less cost and labor

than mechanical weed control methods. In addition to these advantages, flaming also has some disadvantages. The application rate is slower than the use of herbicides. The selectivity between the cultivated plant and the weed is low. Mechanical weed control methods and flaming method have almost the same capacity. Another disadvantage is that the use of propane or diesel fuel causes greenhouse gas (CO<sub>2</sub>) emissions to the atmosphere. According to a study, 60 kg ha<sup>-1</sup> propane gas causes 188.9 kg ha<sup>-1</sup> CO<sub>2</sub> emission to the atmosphere. High fuel consumption and greenhouse gas emissions are the disadvantages of the flame method (Ascard, 1995).

### **3.2. Hot Water**

Hot water applications are used as a popular technique to control weeds that grow between stones on roads and pavements (De Cauwer et al., 2015). Flaming is a safe, economical and simple thermal weed control method that does not have harmful effects like microwave weed control methods. It is an effective weed control method that suppresses perennial weeds that prevent the emergence of many annual weeds. The effectiveness of this technique depends on the high weed density as it increases the penetration ability. In European countries, hot water application is considered as a successful method among sensitive weed management strategies due to its high success rate.

#### **3.2.1. History**

In the early 1900s, hot water application was successfully defined as a weed control method in many countries. This method was defined as

eliminating the disadvantages of flaming applications such as flame damage and energy consumption. A commercial machine named Aqua-Hot has been developed in the USA in order to control weeds. This machine was able to compete with the effectiveness of glyphosate applications in the control of many annual and perennial weeds. Later, a similar equipment was developed in New Zealand to be used in landscape areas called "the Waipuna system". This equipment has been developed by adding hot foam to the system in order to be in contact with weed species for a long time, to be used in the fight against single and perennial weeds in the long term. Today, equipment called "H<sub>2</sub>O Hot Aqua Weeder" is used especially in vegetable growing in Denmark and the Netherlands for weed control applications with hot water (Groeneveld et al., 2002). Compared to the flaming application, the hot water holds more in the plants. However, energy efficiency is higher and these systems need to be developed to reduce water temperature loss. In this context, energy savings and application capacities have been increased by adding digital cameras to hot water spray equipment. A few years ago, a system called 'Wave Machine' was developed with precision application in the Netherlands (Rask & Kristoffersen, 2007).

### **3.2.2. Mechanism of Action**

Hot water dissolves the cuticle membrane in the plant leaves, washing the cell structure and causing the water to not hold in the plant within a few hours or days. This technology is equally effective on both germinating plants and mature plants. It has been observed that factors such as weed growth stage, exposure time, water droplet size, wetting

agent, water temperature, day length variation in thermal sensitivity and water flow affect the success of hot water applications in controlling weeds.

### **3.2.3. Effect on Growth and Reproduction of Weeds**

In many studies, they stated that hot water applications control annual and young perennial weeds in a way to compete with glyphosate. Many non-chemical weed control methods require reapplication due to re-emergence of weeds. Therefore, some hot water applications may require 3 to 5 applications during the growing season to keep weeds under control. Since the area where the hot water can hold is less in monocotyledon plants, it is difficult to control with hot water application. Hot water application at an energy dose of 589 kJ m<sup>2</sup> *Lolium perenne* L., *Festuca rubra* L., *Taraxacum officinale* F.H. wigg. and *Plantago major* L. was found to be effective in controlling weeds. Due to their upright growth, monocotyledon weeds are less sensitive to hot water. In organic farming, approximately 80% mortality rate was observed in broad-leaved dock (*Rumex obtusifolius* L.) in 90 °C hot water application (Latsch & Sauter, 2014).

### **3.2.4. Advantages and Disadvantages**

Compared to chemical weed control, hot water applications do not pollute groundwater, soil and air, and do not cause potential harm to humans and wildlife due to pesticide residues. On the other hand, hot water applications are broad-spectrum like many chemical herbicides that also affect beneficial soil organisms. For a large-scale application,

the cost of hot water treatment is not low; therefore, some use propane gas flaming or infrared heat for direct weed control is more energy efficient and preferable to hot water.

### **3.3. Saturated Steam**

High temperature steam is used as a thermal weed control method. Weed control by steam is an effective, fast and sustainable method used to control weeds, especially in non-agricultural areas. For the last few years, this technology has been used in areas where vegetation needs to be completely destroyed (water channels, runways, etc.). Also used before planting of high value crops.

The main disadvantages of steam application are cost and undesired effects against microarthropods, microorganisms and the natural soil microflora, especially, nitrifying bacteria which are more sensitive to heat treatments (Gay et al., 2010).

#### **3.3.1. History**

This method was previously used to sterilize the soil under laboratory conditions and to control both weeds and diseases. Due to the harmful effects of methyl bromide, researchers have started to work on the hot steam method. In the early 1900s, it was determined that hot steam could control weeds, but it could not be used in practice as an effect. Later, it started to be used effectively in agriculture and forest areas. Different mobile steaming equipment is used in multiple tunnels and agricultural areas to control weeds and pathogens.

### **3.3.2. Mechanism of Action**

In this method, a mixture of evaporated water and steam is sprayed on the weeds. Steam has high energy density with high heat transfer property. Due to gas technology, the heat intensity is 1000-2000 times higher compared to the flame. Therefore, wet steam immediately increases the temperature of the tissues on the plant surface, causing a destructive effect. Many different hot steam applications have been developed for use in open areas and greenhouses. The efficiency and fuel consumption of this system largely depend on weed type, growth period, soil structure (Malender & Kristensen, 2011), application time and temperature. In recent studies, it is aimed to make hot steam applications with less labor. Some of these developments are machines such as the belt steamer and the Ecostar SC600 with blades, and the use of active compounds such as KOH and CaO to increase soil temperature. In open field cultivation, different environmental factors such as air temperature, soil structure and moisture affect the sensitivity of seeds to hot steam.

### **3.3.3. Effect on Growth and Reproduction of Weeds**

While a single steam application can control many annual and early perennial weeds, two applications are required for effective control of mature perennial weeds. This method does not affect the underground parts of the weeds. However, since it damages the above-ground parts of the plants in repeated applications, it causes the plants to re-emerge and the stores in the plant roots decrease, causing the plants to die. It was determined that many weed species that were germinating in a short

time using superheated steam (400 °C) died. According to a studies 3,200 kg ha<sup>-1</sup> of hot steam application significantly reduced weed biomass with controlled 90% of weeds such as *Chenopodium album* and *Amaranthus retroflexus* and was comparable with glyphosate (560 g ei/ha) (Kolberg & Wiles, 2002).

Hot steam applications made while the weeds were in the seedling stage caused a decrease in the biomass of the weeds. However, the biomass reduction in cheatgrass (*Bromus tectorum* L.) was possible when the hot steam applied during the flowering period. According to some studies, an increase in soil temperature of approximately 70 °C reduces the emergence of weed species by 99% (Melander & Jørgensen, 2005). Similarly, in field trials, it was found that tape hot steam application at a maximum of 86 °C reduced the one-year weed population by 90% (Hansson & Svensson, 2007). Damp water steam application for controlling weeds in barley, maize and onion field and as a result 98% of weed seeds were destroyed and suggested as a weed control method for organic agriculture (Kerpauskas et al., 2006). The effect of soil steaming with KOH and CaO as an activating compound on weed suppressing were evaluated. Three winter annuals, foxtail (*Alopecurus myosuroides* Huds), chamomile (*Matricaria chamomilla* L.), wild radish (*Raphanus raphanistrum* L.), and four spring annuals, redroot amaranth (*Amarathus retroflexus* L.), jungle rice (*Echinochloa crus-galli*), wild buckwheat (*Fallopia convolvulus*), and green foxtail (*Setaria viridis* L.) were tested for seed bank and seedling emergence on the field and controlled environment. While *A. myosuroides* was the

most sensitive species (77% reduction), *E. cruss-galli* and *M. chamomilla* L. were the least sensitive (Barberi et al., 2009). Surface steaming applied at a depth of 0-7 cm has been found to be effective because it controls weeds up to 100%. However, steam applications at a depth of 7-14 and 14-21 cm were found to consume up to 95% of weed seed banks in the deep soil layers (Peerzada & Chauhan, 2018).

### **3.3.4. Advantages and Disadvantages**

In the hot steam weed control method, the heat can be directed more precisely to the unwanted vegetation. In addition, hot moisture-laden air can be applied on the targeted plants, there is no risk of fire such as flame. It has less effect on the cultivated plant and beneficial insects. Compared to hot water application, it consumes less water and retains more on vegetation. Hot steam application provides more effective control with very high temperature and short application time. In addition, since it has a high heat transmission coefficient compared to hot water, it is effective by providing more heat transmission during contact with the plant. On the other hand, it is easier to evaporate than water, so heat is lost very quickly. The biggest disadvantage of this method is that it is costly and requires labor due to its high fossil fuel consumption. Investment costs for hot steam equipment are very high, and environmental and weather conditions affect the efficiency of use. Although steam treatment can be used both outdoors and in greenhouses, it has limited application for preserving high-value crops. In addition, the hot steam method is difficult to apply to combat weeds in major agricultural products such as paddy, soybean and wheat

cultivated in large areas. Therefore, weed control with hot steam requires technological innovations such as being applicable in large areas, fast operation and low costs.

### **3.4. Hot Foam**

Hot foam application is a non-toxic weed control method and can be used to control many weed species. It is a promising method as it is practically applicable in sustainable agricultural production including horticulture and organic agriculture. Besides the fact that the hot foam application can be used in agricultural areas, it is also used in the control of some weeds in non-agricultural areas due to the low water consumption and not causing air contamination. Instead, hot foam is mostly used in the control of bacteria, fungi and other important diseases in the soil.

#### **3.4.1. History**

Hot foam technology for weed control was first patented in 1995 and was developed by Weedling WeedingTechnologies Ltd., GB company (James et al., 2009). The patentee company stated that the system was successful as the hot foam application on the vegetation melted the cuticle layer of the plants. However, in order for the system to work effectively, the temperature must be kept constant for a long time. Later, some researchers developed new methods for weed control with hot foam and added various apparatus to the system.

### **3.4.2. Mechanism of Action**

In this method, it is based on the application of compressed hot air on the plant by foaming it with various foaming agents. The hot foam melts the waxy cuticle on the plant surface and causes the death of the plant. Coconut and corn sugars are used as foaming agents. In Sweden, alkyl polyglucosides are used as a foaming agent for hot foam application with hot water in weed control on train maintenance stations and railway tracks.

### **3.4.3. Effect on Growth and Reproduction of Weeds**

In this method, it is useful to control weeds because the hot foam can stay on the plants for a long time. This method has advantages such as being safe, low cost, fast, less affected by air temperature changes, and high application sensitivity. Application of hot foam application 3 times in 1 month killed 50% of *Crassula helmsii* weed, this result showed similar results with glyphosate application (Bridge, 2005).

There is limited information on weed seed production, development and growth of hot foam application. More work needs to be done on this topic in the future.

### **3.4.4. Advantages and Disadvantages**

The hot foam method is the most effective thermal weed control method compared to thermal weed control methods such as flaming, hot air, and hot steam. It is more advantageous than hot water application due to less water consumption and more heat transfer time. More temperature

transfer time, selectivity in weed control, and lower costs through the use of lower temperatures. This method does not have any negative effects on soil properties (Cederlund & Börjesson, 2016).

Thermal weed control methods have many advantages as well as disadvantages and many aspects that need to be developed in the future. Integrated weed control is recommended by using thermal weed control methods together with some other non-chemical weed control methods. In order for this method to be usable in different agricultural systems (organic agriculture, traditional agriculture, etc.), it must be applied practically and energy costs must be reduced.

## **CONCLUSION**

Weed control in crop production require higher costs than disease and pest control. Because while diseases or pests appear occasionally, weeds emerge relatively continuously. Despite of billions of dollars spent on weed control, they cause significant reductions in crop yield (13.2%). In order to reduce the negative consequences of the increasing use of chemicals in agricultural areas today, researchers have started to work on non-chemical control methods to control weeds. These control methods are mechanical, physical, methods applied directly on weeds (rays, radiation, flaming, etc.), or indirectly using energy (such as solarization, mulching). Alternative control methods are not considered sufficient to minimize the harmful effects of chemicals. In addition, it is important that the methods are applicable, sustainable and economical. While some of the weed control methods tried to be summarized in this paper have been put into practice especially in

developed countries, research on some of them is still ongoing and there are many more methods that need to be optimized. All these applications, alone or in combination with other methods, significantly reduce the use of herbicides. In order to prevent herbicide resistance, which is one of the most important problems in countries where herbicide use is intense, great importance is given to these alternative methods. In Turkey, the number of studies on this and related topics has increased in recent years. Increasing environmental awareness and the increase in consumers' sensitivity and awareness on this issue will increase also the demand for these and similar methods in the near future.

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## **CHAPTER XV**

### **HYDROPONIC GROWING FOR CUT TULIPS**

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## INTRODUCTION

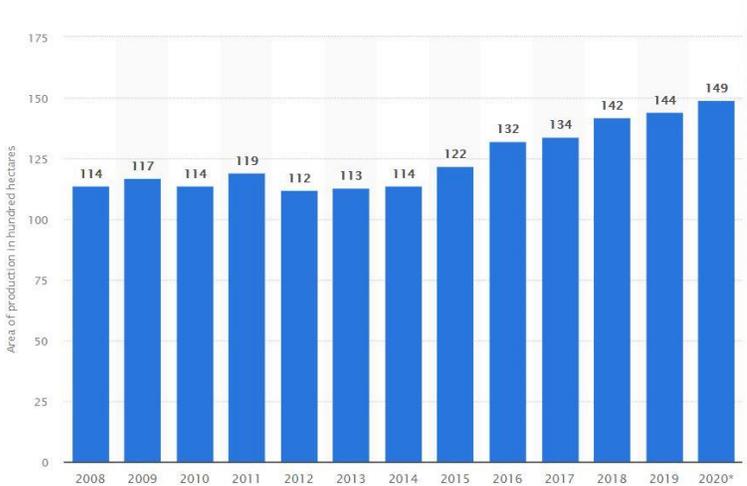
With a 400-hundred-year-old horticultural background the tulip continues to hold its place as one of the most favoured ornamental plants (Van Tuyl & Van Creij, 2005). For centuries a wide range of visually attractive cultivars were obtained through hybridization (Van Scheepen, 1996). Therefore, various colours, shapes and lengths were utilized both in landscapes and floriculture (Salman et al., 2015).

As a reminder of spring the tulip is well regarded among bulbous plants with more than 3000 registered cultivars under 15 sections depending on the flower shape, size, and blooming time (Anonymous, 2021a). Within the plant kingdom, tulips belong to the lily family (Liliaceae). Many bulbous plants such as *Lilium*, *Hyacinthus*, *Muscari*, *Ornithogalum*, *Fritillaria*, etc. belong to the Liliaceae. Characteristics of this family are flowers which bear 6 petals and 6 stamens seedpods that are formed later above the base of the flower (Anonymous, 2021b). Tulip cultivars that were favoured the most were obtained from *Tulipa gesneriana*. Tulips are often classified as single early, double early, triumph, Darwin hybrid, single late, lily-flowered, fringed, viridiflora, Rembrandt, parrot, double late, kaufmanniana, fosteriana, greigii and multiflowering in accord with early, middle or late flowering time, single or double blooms, fringed or simple edged petals. The length of the tulip stems various between 10 to 80 centimetres (Salman et al., 2015).

Tulips bloom in spring in nature. If the plant is forced flowers appear earlier. Flower forcing is an essential part of planning in tulip production. Forcing determines the starting period of propagation and the release date for marketing.

Bulbous plant production and floriculture hold an important place in world trade. A large part of tulip production is used for floriculture compared to relatively small usage in landscape or in pots. The total area of ornamental bulb production in 2005 covers an estimated 32.200 ha in the world (De Hertogh et al., 2012); tulips, lilies, daffodils, hyacinths and gladioli are the most sought-after species among others. The Netherlands takes the lead with 65% of production area (Le Nard & De Hertogh, 1993); The United Kingdom, France, China, and The United States of America follow (Buschman, 2005; Janick, 2010). The total tulip production in the Netherlands increased between 2008 – 2020 and reached 14.900 ha in 2020 (Figure 1) (Anonymous, 2021c).

The tulip plant is mainly used for landscape purposes in Turkey rather than in floriculture, and a large number of bulbs are produced in Konya province. Most of the cut tulips in the Turkish market have been imported from the Netherlands. The length of blooming period in spring is between 20 to 30 days depending on the soil type and the climate of the planting location (Salman & Wallace, 2020). On the other hand, in a controlled environment, the flowering length could be extended from December to April (Salman, 2013). The tulip production for floriculture could be conducted in fields or in greenhouses with either hydroponic or sand trays (Gill et al., 2009).



**Figure 1:** Area Used for Production of Tulip Bulbs in the Netherlands 2008-2020 (Anonymous, 2021c)

## 1. HYDROPONIC CUT TULIP PRODUCTION

### 1.1. Choosing Bulbs

The right bulb choice for floriculture firstly depends on the bulb size which is determined by the diameter of the bulb in centimetres. For the best quality of flowers, the bulb diameter should be 12 centimetres and above (12+) (Figure 2). In some cultivars 11/12 cm bulbs are accepted (Granneman, 2008). Bulbs with lower figures result in a smaller flower size and a shorter stem length which is not desirable for cut flowers. Secondly, the bulbs must not contain any pests or diseases and they should not bear physical damage (Salman, 2013).



**Figure 2:** An Example of 12+ Centimetre Tulip Bulbs (Original by Salman)

## 1.2. Precooling

Following the harvest, tulip bulbs are left in storage facilities with fresh air circulation at 20 °C. The flower formation starts to occur in 8 weeks' time (Granneman, 2008). Depending on the cultivar, the formation is complete within 2 to 4 weeks. The bulbs must go through a cool period to develop the sufficient stem length and then the flower. In cold climates this is achieved in the field naturally. If the bulbs are exposed to cold before the winter they can bloom earlier. Therefore, it is necessary to precool the bulbs to obtain the desired flower structure. The ideal temperature is 9 °C and below (Gill et al., 2009). Higher temperatures result in rapid root development, shorter stem length and a longer production period. The tulip bulbs can be obtained by producers either precooled or not. The precooled bulbs must be planted immediately. If the bulbs are delayed, they must be stored between 4-7 °C for only a short time. On the other hand, non-precooled bulbs can be planted after being stored in the dark for 12-14 weeks at 5-9 °C.

### 1.3. Hydroponic Propagation

The Netherlands propagates roughly 50% of its tulip bulbs in a hydroponic environment. For this purpose, specially designed reusable pin trays are preferred (Gill et al., 2009). The pins on the bottom of the tray act as an anchor and prevent the bulbs from rolling over during the development period (Figure 3). While the bulbs are located by hand, the pins must not pierce the centre of the bulb but rather around the outside of it (Figure 4). Right after the bulbs are settled in the tray it must be filled with water and moved to the rooting chambers. The bulbs are to remain in chambers set at 5 °C for 1 to 3 weeks (Figure 5). It must be noted that if the temperature is set above this figure, *Penicillium* could occur in bulbs. The presence of water initiates the root development. The drainage holes around the sides of the tray prevent the water from interacting with the rest of bulb (Gill et al., 2009). The amount of water required by the bulb increases as the plant develops. The level of the water should be checked regularly and be kept sufficient to always cover the roots. Otherwise, the roots turn a brown colour, and the plant development is impaired. When the trays are replenished it is necessary to give more than the required level to refresh the water. Yet if the greenhouse floor is kept wet all the time *Botrytis* may occur in the environment.



**Figure 3:** An Example of the Pin Tray and Pin Pierced Bulb (Original by Salman)



**Figure 4:** Tulip Bulbs Located in Pin Trays by Hand (Original by Salman)



**Figure 5:** Tulip Bulbs in Rooting Chambers (Original by Salman)



**Figure 6:** An Example of Rooting Tulip Bulb (Original by Salman)

#### **1.4. Fertilization**

As the tulip bulbs contain many natural elements fertilization must be executed moderately. Excessive application can result in shorter stem lengths and a lower market value. The bulbs reach their harvest period rather rapidly therefore slow-release fertilizers are not advised. In a hydroponic environment the fertilizers are applied after the root development occurs and the shoots have reached 5-6 cm. Calcium nitrate and potassium nitrate at a 2:1 ratio are added to the trays. The solution's EC value should be set to 1.0 – 1.5 and the pH value to 6.0 (Miller, 2002). The ideal greenhouse temperature is 17-18 °C for optimum growth. During the cooler periods of production this temperature could be lowered by 1-2 °C. With increasing outdoor temperatures, a shade should be provided in the greenhouse (Armitage, 1991; Armitage et al., 1990). The humidity (RH) is to be kept around 80 – 85% with plenty of air circulation.

### **1.5. Harvest**

The tulip bulbs reach the harvest period between 26 to 60 days in accord with the cultivar's genetic characteristics. During this time the greenhouse temperature must be kept under 16 °C and the day and night temperature oscillation must be kept under control. Also, as the tulip plant is not fond of bright light shade must be provided to obtain the right plant colour.

Harvest is carried out when the flower colour starts to appear, and the flower shoots are still enclosed (Figure 7). The cultivar Darwin requires more colour to show before the harvest (Gill et al., 2009). For the harvest, the tulips are completely removed from the trays to separate the bulbs from the stems and form flower bunches. The separated bulbs are discarded, not to be used again (Figure 8). If the plants reach harvest time yet need to be waited on, they should be kept in cold storage with the bulbs intact. The bulb – stem separation can be done by hand or machinery (Figure 9).

The harvested tulip flowers are kept in cold water (1-5°C) for 30 to 60 minutes before transportation. This duration could be extended up to a week if needed or they can be kept at 2 °C in dry conditions. The tulip bundles are prepared at the same length (Figure 10) yet they can grow up to 15 cm more after being located in containers (vases etc.) (Gill et al., 2009).

Tulips could also be grown in sand filled trays or in fields. Some cultivars are regarded as being more suited to sand production (Figure

11). But growing the plant in a hydroponic environment is significantly advantageous.



**Figure 7:** Tulips ready for Harvest (Original by Salman)



**Figure 8:** Discarded Bulbs after Stem Separation (Original by Salman)



**Figure 9:** Size Classification of Tulip Bundles (Original by Salman)



**Figure 10:** Arranging Tulip Bouquets (Original by Salman)



**Figure 11:** Sand Filled Tulip Trays for Cut Flower Production (Original by Salman)

The advantages of hydroponic propagation are (Miller, 2002),

- Absence of soil cost,
- Rapid growth period,
- Easy harvest,
- Reusable trays,
- Lower energy costs,
- Clean propagation product and environment,
- Effective management of pests and diseases
- Less usage of pesticides
- Multiple usage of rooting chambers.

On the other hand, the hydroponic propagation holds some disadvantages (Miller, 2002),

- Cost of pin trays,
- The need of rooting chambers,
- Lighter tulip bulbs, taller body,
- The risk of tilling over of plants,
- The risk of root infections,
- The cost of disinfection.

## **CONCLUSION**

From 2010 more than 200 tulip cultivars have been examined for bulb yield and flower quality in Ege University, Bayındır Vocational Training School, and the most suitable tulip types were identified for the area (Figure 12 and Figure 13).

The area bears the characteristics of the Mediterranean region which proves to be advantageous for the early harvesting of tulip bulbs. Having an early harvest of cut tulips is a desirable outcome for marketing purposes. Similarly, The Netherlands offers early harvested tulip flowers raised in Italy, France and Spain which bear the same climatic characteristics as Izmir, Turkey. This situation indicates that there is an opportunity for Turkey to grow and export early harvest cut tulips to Europe.



**Figure 12:** Tulip Research Fields in Bayındır, İzmir – 1 (Original by Salman)



**Figure 13:** Tulip Research Fields in Bayındır, İzmir – 2 (Original by Salman)

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