Ornamental Plants in Different Approaches

Assoc. Prof. Dr. Arzu ÇIĞ

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CONTENTS

Ornamental Plants, which have a different place among plant groups, have started to take their deserved place in our lives. The ornamental plants we use to soften the increasingly stressful living conditions, except for their cultivation and benefit in food, textile, medicine, cosmetics, industry fields, bring us closer to nature, especially in this technological-digital age. In fact, we can say that there are many unknown aspects of these beautiful ornamental plants that we see at home and indoors, on our balconies, parks and gardens, and they touch us in areas we have never known in our lives.

In this book, these areas are tried to be addressed from different ways. These studies cover the botanical and phenological characteristics of some ornamental plants, their phyto-chemical content, ecological characteristics, fertilization, disease-pest-weed control, growing conditions, production techniques, usage areas, health benefits and the place of this sector in our country.

I would like to thank all of our authors, who were by our sides with their studies and support, in this book, which includes works that are very valuable both academically and scientifically, and I hope that everyone who is interested in these issues find what they are curios about in this book and that the information in it will benefit everyone.

Sincerely Yours

Arzu ÇIĞ

CHAPTER 1

DOUBLE FLOWER TRAIT IN ORNAMENTAL PLANTS: FROM HISTORICAL PERSPECTIVE TO MOLECULAR MECHANISMS

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INTRODUCTION

The transformation change in nature that occurs in the DNA or RNA sequence in the living genome is defined as a mutation. Changes in the outer appearance of a mutated creature can be shown in different ways. One of the results of the transformation change occurring in the genome of plants is stratification. The stratification feature is very rare and rarely occurs in nature. As the interest in plants with double flowers increases, researches on the feature of stratification in the natural environment, even though it is rare, has gained importance.

Double flowers are necessarily favorites with almost every one, being, with few and rare exceptions, much more beautiful than the single forms of the genus, and also, in the majority of cases, evincing the exercise of that skill, which, when attended with favorable results, is always so pleasing.

1. DEFINITION

The formation of flowers, which is the most remarkable organ of plants in the stratification issue, develops in a mysterious way. Each flower starts to be formed mainly as a small cluster of undifferentiated cells within the bud; then, it changes into a complex structure that includes different organs with defined functions.

Each organ in a flower has its own specific cell type, group, and function. The flower shape is formed during the embryonic stage. While some cell groups develop through the cell type they already have, some cell groups can also develop in a different way. Plants that develop in a different way are defined as "mutants". Stratified flowers, which are part of mutant plants and formed naturally, are of specific importance. Although wild plants in nature generally have plain flowers, some kinds have double flowers. The term 'Double Flower' is actually a type of flower which is grown anomalous, and is often used to describe flower varieties with extra petals. Due to the mutation of three genes that have a function in flower formation, as a result of the transformation of early female organs into petals, a double flower is formed.

The double flower feature is usually named as 'fl.pl.' which is an abbreviation for the Latin word "flore pleno" meaning "full flower" (Baytop, 1998). Since this feature in plants has a gene character and the morphological characteristics of the recorded types support it, it was seen as a variation for the species and it was accepted at the form level in the botanical classification.

2. FEATURES AND USAGE

The stratification feature that takes place in nature during the evolution of the species, over long periods of time and by themselves, is applied in current days in different artificial methods. Particularly, it is used to increase diversity by using physical and chemical factors.

In recent years, mutation studies carried out for stratification in plants have become increasingly important. The diversity is increasing due to the increasing demand especially in the field of landscape architecture and cut flowers. For this purpose, most of the genes that generate the male organs are disinclined in the cells of the flower structure of the plant subject to application, and only the genes that encourage petal formation are allowed to grow.

The petals of species with double flowers can be in different numbers depending on the development process of the plant. Double flowers are grouped in three groups according to the number of petals. Flowers with 9-16 petals are grouped as "semi-layered", flowers with 17-25 petals are named as "fully laminated", and those with more than 25 petals are defined as 'multi-layered' (Phillips & Rix, 1988).

In some blooming species, the size and color of the outer and inner petals are different. Layered blooming plants bloom later than normal blooming plants and with a longer blooming period.

In some Double flower varieties, the plant is not able to produce anymore as all reproductive organs turn into leaves. In order for these species to continue their production cycle, they must be reproduced by “Artificial Vegetative Propagation”. In some species that are fertile and due to the difference in the number of petals, some flowers may produce a small number of seeds which can be used for reproduction even if the current plant does not continue to grow.

Species with double flowers are stronger with more durable growth than other species. In addition, these species are taller and their leaves are larger.

The first double flower plants to be cultivated are rose (*Rosa* L.), carnation (*Dianthus*), and *Camellia* species. These species are particularly popular in the ornamental plant market. Pomegranate (*Punica granatum* L.), which is considered as fruit trees and used as an ornamental plant for decorative and aesthetic purposes, is an exemplary special plant among plants with double flowers (Meyerowitz et al., 1989; Cothran, 2003).

The ornamental pomegranate has a carnation shaped flower. It blooms from the early season of spring to autumn. It has fiery red-orange flowers that do not turn into pomegranate fruit. The plant grows fast and is easy to cultivate. Due to these characteristics, it can also be grown in pots as a house plant.

Double flowering plants are used both in the garden, in pots, and in cut floriculture due to their strong and durable structure, as well as their large and wonderful leaves and flowers.

3. THE HISTORICAL BACKGROUND

The Double flower structure, which was defined about two thousand years ago, is among the oldest documented flower abnormalities.

It is known that Chinese roses, which are considered to be the ancestors of hybrid tea roses, have been grown since 1000 BC. At the present time, the most commonly grown varieties of layered roses are considered to have this feature. Theophrastus of Lesvos (371-287 BC), an ancient scientist who also made researches in the field of botanical science, mentioned the feature of double flowers in his book on plants. The philosopher and writer Gaius Plinius Secundus (23-79) described roses with a Double flower structure in his famous work "Natural History" (Naturalis Historia). In 750 AD, the peony (*Paeonia arietina* G. Anderson) plant was known and cultivated in China (Anderson, 1818; Meyerowitz, et al., 1989).

It is accepted that the layered blooming plants were not common in Europe when they were grown in Asia and Mesopotamia. The Europeans, who came to Anatolia and Mesopotamia during the Crusades, got to know about the varieties and the features of the Double flowers in these regions. Among the first plants that were introduced to Europeans during that period are "*Rosa alba* L. var. *semiplena* Hort." and "*Rosa hemisphaerica* J. Herrm. var. Plena Hort." (Baytop, 2001)

Throughout history, the layered blooming plants, which gardeners and botanists have been interested in, have also attracted the attention of artists and have been the subject of many visual artists' works. The Renaissance period is the period, especially in Europe, when double flowers became more important and started to be grown in gardens.

The Flemish Physician Rembert Dodoens (1517-1585), also known as a botanist, published his writings on the Double flower in 1568. The British Botanist and Priest John Gerard (1545–1612) made sketches of natural double flowers in 1597 (Wijnandsf, 1994).

Lilpar (*Caltha palustris* L.), also known as the 'Marsh Marigold', became among the admired ornamental plants in a very short time after it was discovered in Austria at the end of the sixteenth century (Wijnandsf, 1994).

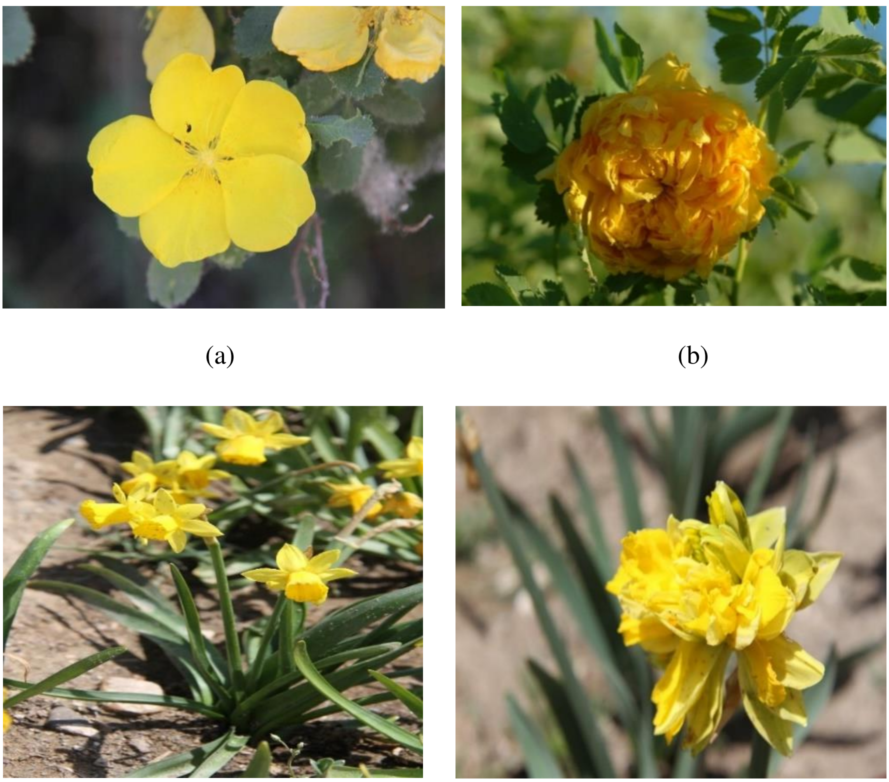
In double flowers, stamens have been transformed into extra petals for a fuller, showier bloom. The lack of pollen means pollination cannot occur and the flower remains open for longer, waiting. The use of layered blooming plants is a recommended solution to the pollen allergy problem that emerges and spreads with urbanization in recently. Layered blooming plants are preferred in landscape designs due to the fact that they have low allergy features as they don't emit pollen or emit very little pollen.

For this reason, many spontaneous double-flower ornamental plants were kept and propagated for landscape architecture. In recent years, some species have been introduced to matured flowering with gene silencing therapies. The petunia (*Petunia hybrida*) plants, and peony (*Ranunculus asiaticus* L.) are good examples of this introduction.

Since double flowering plants do not contain pollen and nectar or have very little, they are not particularly important for wildlife that feed on nectar. Therefore, laminated flowering plants are not recommended for use in rural areas.

4. SOME DOUBLE FLOWERING PLANTS

Double flowers are much more beautiful than the single forms of the genus so favorite's with almost all gardeners. Below is a list of some species that have double flowers in nature, including examples of commonly used ones in recent days (Table 1). Figure 1 shows single and double flowering *Narcissus pseudonarcissus* and *Rosa hemisphaerica* species as examples.



(a) (b)

(c) (d)

Figure 1: (a) *N. pseudonarcissus* has single-flower, (b) *N. pseudonarcissus* has double flower, (c) *R. hemisphaerica* has single-flower, (d) *R. hemisphaerica* has double flower (Original by Alp)

Table 1: Ornamental plant species have double flowers

Double flowering plant species
Achillea ptarmica 'fl.pl.'
Adonis amurensis 'fl.pl.'
Agrostemma coronoria 'fl.pl.'
Alcea rosea 'fl.pl.'
Arabis alpina 'fl.pl.'
Begonia x tuberhybrida 'fl.pl.'
Bellis perennis 'fl.pl.'
Caltha palustris 'fl.pl.'
Calystegia hederacea 'fl.pl.'
Chamaemelum nobile 'fl.pl.'
Clematis florida 'fl.pl.'
Coreopsis grandiflora 'fl.pl.'
Coreopsis grandiflora 'fl.pl.'
Crataegus laevigata Rosea 'fl.pl.'
Crataegus laevigata Rosea 'fl.pl.'
Delphinium grandiflorum 'fl.pl.'
Dianthus plumarius 'fl.pl.'
Eranthis hyemalis 'fl.pl.'
Ficaria verna 'fl.pl.'
Galanthus nivalis 'fl.pl.'
Geum 'fl.pl.'
Helianthus annuus 'fl.pl.'
Hemerocallis fulva 'fl.pl.'
Hibiscus mutabilis 'fl.pl.'
Iris pseudacorus 'fl.pl.'
Kerria japonica 'fl.pl.'
Lilium lancifolium 'fl.pl.'
Narcissus odorus 'fl.pl.'
Narcissus poeticus recurvus 'fl.pl.'
Paeonia arietina 'fl.pl.'
Paeonia officinalis 'fl.pl.'
Papaver rupifragum 'fl.pl.'
Philadelphus mexicanus 'fl.pl.'
Portulaca grandiflora 'fl.pl.'
Primula vulgaris 'fl.pl.'
Prunus avium 'fl.pl.'
Prunus serrulata 'fl.pl.'
Prunus subhirtella 'fl.pl.'
Punica granatum 'fl.pl.'
Ranunculus acris 'fl.pl.'
Ranunculus ficaria 'fl.pl.'
Ranunculus montanus 'fl.pl.'
Rhododendron fastuosum 'fl.pl.'
Rhododendron sp. 'fl.pl.'
Rosa banksiae 'fl.pl.'
Rosa hemisphaerica 'fl.pl.'
Sagittaria sagittifolia 'fl.pl.'
Sanguinaria canadensis 'fl.pl.'
Saxifraga granulata 'fl.pl.'
Spiraea cantoniensis 'fl.pl.'
Tabernaemontana divaricata 'fl.pl.'
Tanacetum parthenium 'fl.pl.'
Tenuifolia Rubra 'fl.pl.'
Trillium grandiflorum 'fl.pl.'
Ulex europaeus 'fl.pl.'
Vinca minor 'Azurea fl. pl.'
Viola odorata 'fl.pl.'
Zinnia elegans 'fl.pl.'

5. MOLECULAR GENETIC MECHANISM OF DOUBLE-FLOWER TRAIT IN ORNAMENTAL PLANTS

5.1. Molecular Biology of Flower Development: ABC Model

Flower structure is the unique trait in ornamental plants. Flower structure as well as its color were the major breeding targets. Thus, revealing molecular genetic mechanism of flower development was the hot topic in plant molecular biology and breeding. Although homeotic mutations were known for centuries in many flowering plants as mentioned in the first part of the chapter, flower development model called "ABC model" was developed as results of studies performed in model organism *Arabidopsis thalina* and *Antirrhinum majus* in 1991. This model explains roles of three gene families (class A, B and C genes) for development of sepals, petals carpels and stamens. Flowers are originated from flower meristem organized as concentric whorls. While sepals and petals develop in the first and second whorls, stamens and carpels develops in third and fourth whorls, respectively. Class A genes regulate development of sepal in first whorl, Class A and B genes regulate development of petals, Class A and C genes regulate development of stamens and class C genes regulate development of carpers. This flower development model drives two conclusions. (I) class A and C genes have antagonistic effect and (II) class B genes have function with either class A class or class C genes in flower development. Molecular genetic studies reported that while class A genes comprise two genes (APETELA-1 and APETELA-2), Class B genes comprise two genes (APETELA-3 and PISTILLATA), Class C genes comprise one gene called AGOMOUS (AG). Molecular mechanism of ABC gene model was reviewed in more detail by Litt & Kramer (2010); Bowman et al. (2012); and Irish (2017). This chapter is intended to describe molecular mechanisms of a flower homeotic mutation called double flower phenotype.

5.2. Mutational Mechanisms of Double Flower Phenotype

5.2.1. Loss of Function Mutation in AGAMOUS (AG) Gene

Loss of function mutation in AG gene which belong to class C genes is responsible for double flower phenotype in *Arabidopsis thalina*. Primarily two loss of function alleles (ag-1 and ag-2) of AG gene were identified in *Arabidopsis thalina* (Bowman et al., 1989; Yanofsky et al., 1990). Subsequently, three mutation in APETALA2 loci (p2-2, ap2-8 and ap2-9) and two new mutations in PISTILLATA locus (pi-2 and pi-3) and third alle of AG gene (ag-3) were discovered by Bowman et al. (1991). Thus, identification of these genes and alleles leaded to propose ABC model in flower development and demonstrated the function of AG gene. After that homologs of AG gene were identified in many ornamental plants and these studies showed that loss of function mutations in AG gene is mainly associated with double flower phenotype. The first homolog of AG gene was identified in *Antirrhinum* (Bradley et al., 1993). Afterwards, AG gene homolog DUPLICATED (DP) gene in Japanese Morning Glory leaded DF phenotype (Nitasaka, 2003). Another homolog of AG gene called LLAG1 in lily (*Lilium longiflorum* Thunb) was identified (Benedito et al., 2004). Also, AG homolog was identified in Japanese Apricot (*Prunus mume* Sieb. et Zucc) (Hou et al., 2011). Other studies showed that AG gene was conserved in more than 20 flowering plants (Zahn et al., 2006; Liu et al., 2010: Hou et al., 2011). Also, several studies reported that loss of mutations in AG homologs were mainly associated with DF in several species including *Thalictrum thalictroides*, *Japanese gentian* and *Matthiola incana* (Galimba et al., 2012; Nakatsuka et al., 2015; Nakatsuka & Koishi, 2018). In a recent study performed by Sasaki & Ohtsubo (2020), two homologs (*TfPLE* and *TfFAR)* of AG gene were silenced using RNA interference (RNAi) and CRISPR/Cas9-mediated genome editing technologies. As results, silencing both genes produced multi-petal phenotype. All these studies showed that AG homologs are promising target for increased petal number breeding in ornamental plants. Especially currently availability of practical genome editing technologies such as CRISPR/Cas9 provide great opportunity to create loos of function mutation in AG homologs in ornamental plants. Some of ornamental double flowering plants generated by genome editing technologies are listed in Table 2.

Table 2: Double flowering plants generated by genome editing technologies

Plant species	Genome editing technology	Reference
Torenia fournieri	RNAi and CRISPR/Cas9	Sasaki & Ohtsubo, (2020)
Pasqueflower (*Pulsatilla koreana*	Chemical mutagenesis	Lee et al., (2010)
Cyclamen persicum (cyclamen)	Chimeric repressor silencing technology	Tanaka et al., (2013)
Petunia	Virus-induced gene silencing	Noor et al., (2014)

5.2.2. Loss of Function Mutation in AP2 Gene

Loss of function mutation in AG homolog associated with DF phenotype in roses was reported by Dubois et al. (2010) for the first time. Although downregulation of AG homologs seems to be responsible for DF phenotype, molecular mechanism leads DF is uncomplete due to non-colocalization of AG gene with QTL control petal number and unaffected expression of the gene in roses have DF (Bendahmane et al., 2013; Abdirashid & Lenhard, 2020). Finally, mapping studied performed in roses revealed that one of the two loci (Di2) co-segregated with DF phenotype and the locus was mapped in an interval 150,858 bp in size containing 22 genes. One of these genes was euAP2 transcription factor. Finally, resequencing analysis revealed that AP2 had deletion for binding site for miR172 (Gattolin et al., 2018). The subclade of AP2 genes called *PETALOSA* (*PET*) gene conserved in phylogenetically distant eudicots but not in *Arabidopsis thalina*. After this revolutionary study, several studies identified PET gene homologs and mutants preventing miR172 binding in *R. rugosa, Petunia* and tetraploid tobacco (*Nicotiana tabacum).* PET genes in these plants were clustered in *TOE* clade of AP2 like genes (Abdirashid & Lenhard, 2020).

CONCLUSION

A homeotic mutation called double flower phenotype were known in many ornamental plants for centuries. Ornamental plants have double flower are valuable due to their large and wonderful flowers. Thus, development of double flowering plants is important in ornamental plant breeding. Two genes (**AG** and **AP2)** described in the chapter can mainly explain molecular mechanisms of DF phenotype. These genes are valuable genomic sources to develop cultivars have double flower.

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CHAPTER 2

THE IMPORTANCE OF INDOOR PLANTS

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INTRODUCTION

With the increase in comfort, nowadays people equip living spaces with easier heating and ventilation systems, and live in urban areas with more shopping centers and controlled spaces (Yazgan et al., 2009). Spaces equipped with good conditions must be in completely artificial environments, purified from outdoor and nature, despite all their comfort.

Plants are the design elements of artificial environments that help the spaces to bear traces of natural elements. Among the massive surfaces such as steel structure, glass and concrete, created by modern technology, living plants that carry reflections from nature play an important role in the organization of the interior space. Its seasonal changes, lively and colorful appearance throughout the year add dynamism to the space with both architectural forms and features of leaves, flowers and stems.

With an arrangement made with plants in the interior, the space has a more attractive and different atmosphere by taking advantage of the plant's color, scent, form or measure features (Yazgan et al., 2009). Achieving some functions that can also be performed with inanimate materials with a living object maintains the natural landscape in closed spaces. Although depending on the function of the space, plants are the elements that should be used in interior space arrangement due to their many functional features such as orientation in the space, hiding unwanted objects, softening sharp lines, and ensuring space organization.

Plants have functions such as defining the space, separating different functions from each other, emphasizing, masking, and orienting as well as adding color to their environment (Figure 1-4).

Figure 1: Plants and their volume effect in space (URL-1)

Figure 2: Plants and architectural elements (URL-2)

Figure 3: Plants give texture to the wall surface (URL-3)

Figure 4: Plant cover-screen function (URL-4)

In the exhibition hall or in a hotel lobby, architectural forms can be used to emphasize a desired object (a work of art such as painting, sculpture) or a consultation place. When a dense, evergreen plant is used as a background, the desired artwork can be highlighted. Plants can also be used to warn the user about possible dangers of sudden level change, surface difference (such as pool and floor separation). Indoors, lines that disturb the eyes such as sharp corners and sharp lines can be covered with plants (Yazgan et al., 1999). In public places such as shopping malls, functions such as circulation lines and gathering areas can be directed by stimulating systems made with plants.

In addition, plants have ecological functions as they filter the noise, control acoustics, contain dust, prevent reflection and glare, control light and clean the air by producing O_2.

Plants make the space suitable for the human size in high ceiling spaces. Their textures help delimit and combine large architectural spaces. They are important tools in hiding sharp corners that cause psychological disturbance and softening hard architectural elements.

The contribution of plants to the indoor environment has been proven in the research of NASA (The National Aeronautics and Space Administration). According to the research results; some plants (such as *Ficus benjamin, Chrysanthemun* and *Draceana*) can absorb approximately 90% of the pollutants in the environment within 24 hours. While chemical pollutants are absorbed by the plant leaves, the roots break down the pollutants with the help of microorganisms and then use them as nutrients. A covered plant can hold pollutants in an area of 10 square meters. Since different plant species absorb different pollutants (such as benzene, formaldehyde trichloroethylene-TCE), it is necessary to include mixed species in the environment. Studies have shown that carpet detergent, paint, furniture polishes, floor cleaning detergents, cigarettes are better absorbed by *Hedera* species, *Gerbera*, *Chrysenthemum, Philodendron* species, *Spathiphyllum, Ficus benjamin* (Wood, 1999). In this study conducted by NASA, plants showing optimal development at maturity were used.

Table 1: Amounts of removal of trichloroethylene from the environment

Plant Name	Leaf surface area (cm^2)	Total TCE (micrograms) removed per plant * microgram µg; one millionth of a gram. It refers to the smallest unit showing the weight / mass ratio. 1 / 1,000,000 or $1x10^{-6}$.
Gerbera jamesonii	4.581	38.938
Hedera helix	981	7.161
Dracaena marginata	7.581	27.292
Spathiphyllum "Mauna Loa"	7.960	27.064
Sanseveria laurentii	3.474	9.727
Dracaena deremensis "Warneck"	7.242	13.760
Chamaedorea selfritzii	10.325	16.520
Dracaena massangeatta	7.215	10.101
Dracaena deremensis "Janet Craig"	15.275	18.330

Table 2: Removal of benzene from the environment

Plant Name	Leaf surface area (cm^2)	Total Benzene (micrograms) removed per plant
Gerbera jamesonii	4.581	107.653
Chrysanthemum morifolium	4.227	76.931
Hedera helix	1.336	13.894
Dracaena marginata	7.581	30.324
Sanseveria laurentii	2.871	28.710
Dracaena deremensis "Warneck"	7.242	39.107
Chamaedorea selfritzii	10.325	34.073
Dracaena deremensis "Janet Craig"	15.275	25.968
Spathiphyllum "Mauna Loa"	7.960	41.392
Aglaonema "Silver Queen"	3.085	14.500

The amount of removal of trichloroethylene from closed test tubes by indoor plants during 24 hours is given in Table 1.

The amount of removal of benzene from indoor test tubes by indoor plants during 24 hours is given in Table 2.

The amount of formaldehyde removal from indoor test tubes by indoor plants during 24 hours is given in Table 3.

Table 3: Formaldehyde removal amounts

Plant Name	Leaf surface area (cm^2)	Collected Formaldehyde (micrograms) removed per plant
Hedera helix	1.336	9.653
Dracaena marginata	7.581	20.469
Sanseveria laurentii	2.871	31.294
Chamaedorea selfritzii	10.325	34.073
Dracaena deremensis "Janet Craig"	15.275	48.880
Spathiphyllum"Mauna Loa"	8.509	16.167
Musa oriana	1.000	11.700
Philodendron domesticum	2.323	9.989
Chlorophytum elatum	2.471	10.378
Scindapsus aureus	2.723	8.986
Philodendron selloum	2.373	8.656
Aglonema modestum	1.894	4.382

In addition, the rate of chemicals destroyed by plants in sealed plastic containers in a 24-hour period is given in Table 4.

Commercial forms of all plants were preserved until testing: each test conducted over 24 hours; it was performed at 30 degrees and 125 foot-candles (units of light).

Table 4: Chemicals removed by plants in a 24 hour period

Plant name	Formaldehyde			Benzene			Trichloroethylene		
	Starting (ppm)	Result (ppm)	Removed amaount (%)	Starting (ppm)	Result (ppm)	Removed amaount (%)	Starting (ppm)	Result (ppm)	Removed amaount (%)
Dracaena massangeatta	20	6	70	14	11	21.4	16	14	12.5
Chrysanthemum morifolium	18	7	61	58	27	53	17	10	41.2
Gerbera jamesonii	16	8	50	65	21	67.7	20	13	35
Dracaena deremensis "Warneckei"	8	4	50	27	13	52	20	18	10
Ficus	19	10	47.4	20	14	30	19	17	10.5

The proportion of Benzene destroyed by plants in sealed plastic containers in a 24 hour period is given in Table 5.

Table 5: Benzene removed by plants in a 24-hour period

Plant name	Starting (ppm)	Result (ppm)	Removed amount (%)
Hedera helix	0.235	0.024	89.8
Dracaena deremensis "Janet Craig"	0.432	0.097	77.6
Scindapsus aureus	0.127	0.034	73.2
Spathiphyllum "Mauna Loa"	0.166	0.034	79.5
Aglonema modestum	0.204	0.107	47.6
Dracaena marginata	0.176	0.037	79.0
Sanseveria laurentii	0.156	0.074	52.6
Dracaena deremensis "Warneckei"	0.182	0.055	70.0

The proportion of Trichloroethylene destroyed by plants in sealed plastic containers in a 24 hour period is given in Table 6.

Table 6: Trichloroethylene removed by plants in a 24 hour period

Plant name	Starting (ppm)	Result (ppm)	Removed amount (%)
Hedera helix	0.174	0.155	10.9
Dracaena deremensis "Janet Craig"	0.321	0.265	17.5
Scindapsus aureus	0.207	0.188	9.2
Spathiphyllum "Mauna Loa"	0.126	0.097	23.0
Dracaena deremensis "Warneckei"	0.114	0.091	20.2
Dracaena marginata	0.136	0.118	13.2
Sanseveria laurentii	0.269	0.233	13.4

The amount of removal of Benzene chemical from the environment for 24 hours with and without leaves of some plants is given in Table 7.

Table 7: Benzene amounts removed by plants with and without leaves during 24 hours

Plant name	Starting (ppm)	Result (ppm)	Removed amount (%)
Dracaena marginata			
Leafy	0.343	0.144	58.0
Leafless	0.348	0.175	49.7
Dracaena deremensis "Janet Craig"			
Leafy	0.369	0.077	79.1
Leafless	0.321	0.176	45.2
Scindapsus aureus			
Leafy	0.122	0.040	67.2
Leafless	0.175	0.062	64.6

It has been found that indoor plants requiring low light have a great potential in reducing the pollution that causes pollution in buildings designed for efficient energy use. These plants hold these chemicals by themselves, their roots, and their growing soil (NASA, 1989).

The presence of pollutants such as unburnt hydrocarbons emitted from the heating units, high concentration chlorine gases, sulfur dioxide gases in the presence of swimming pools necessitates the presence of indoor plants. Some plants that contribute to the creation of a healthy and clean environment are given in Table 8 (Lancaster & Biggs, 1998).

Table 8: Useful plants that have a positive effect on the indoor atmosphere

Plants which are useful with their leaves	Plants which are useful with their flowers
Chamaedora elegans *Chlorophytum comosum* "Mandaianum" *Dieffenbachia seguine* "Exotica" *Dracaena fragrans* "Janet Craig" *Dracaena marginata* *Ficus benjamina* "Reginald" *Ficus elastica* "Robusta" *Hedera helix* "Green Ripple" *Nephrolepis exaltata* "Bostoniensis" *Schefflera arboricola* "Compacta" *Spatiphyllum wallisii* *Syngonium podophyllum*	*Begonia* spp. and hybrids *Chrysanthemum* hybrids *Clivia miniata* *Gerbera jamesonii* *Schlumbergera truncata* *Tulipa* hybrids

These plants, which are recommended to be used especially in places such as residences and offices, together with their aesthetic contributions, help to create less stressful spaces with the oxygen they provide. In order for the expected function of the plant to take place, the plant must have certain features.

• The plant must have a certain degree of ecological tolerance. It must be durable enough to tolerate maintenance errors caused by changes in optimum temperature, humidity and other conditions, or due to a certain degree of negligence.

• It should grow well in containers and pots.

• It should be evergreen.

However, in addition to the characteristics of the plant such as high ecological tolerance, suitable species and good development, there are rules that the designer must follow. For example, plants with similar ecological needs should be used where are suitable ecological conditions. The designer should know the requirements of each plant, such as temperature, light, and humidity, and provide suitable conditions for that plant.

In an indoor arrangement made with plants, the space organization can be made with the functions of opening or dividing the spaces in a way that the elements such as walls and roofs are partially fulfilled. Plants can be used as a cover or curtain to create personal spaces (Figure 5-7).

It creates personal spaces in offices by creating a visual obstacle. It increases productivity in working environments by creating visual and physical barriers and reducing social interaction. It also has noise reducing effects.

Figure 5: Plants guide pedestrian circulation (URL-5)

With the locations of the plants and the line they draw, the user group is directed towards the desired target. Thus, pedestrian traffic circulation is regulated.

Figure 6: The restrictive effect of plants in space (URL-6)

It is possible to visually connect two different spaces with plants. Spaces with different functions are separated from each other with the help of plants. It is placed in space as a physical barrier or as a visual separator.

Figure 7: Physical or visual barrier of plants (URL-7)

The psychological perception in the space is carried out with different associations in people created by the form, color and other features of the used plant species (Table 9).

Table 9: Design elements and their effects on human psychology

Human type	Design type	Design element usage type
Relaxation	Sincere, simple thoughts, prompting to think	Flowing lines, focus on curves, stable structures, muted colors White, gray, blue, green
Thinking	Puts the subject in the background, the design is soft, not pretentious, the ratio is not important	Shapes have no message, contrast is not sharp, soft calm colors
Fear	Great proportions	Flying shapes, black and white and contrasting colors
Love	One’s characteristics, close proportions	Close-up with soft round shapes, shades of pink to red
Dynamic movement	Daring shapes	Heavy structural harmony, angular shapes, bright colors (red, yellow, orange)
Joy	Rhythm and dynamic free space	Soft flow areas, patterns, bright colors and shapes
Tension	Unstable shapes, sharp lines	Incompatible colors and glossy surfaces within the same composition
Horror	Feeling of imprisonment	Dark, obscure abnormal monochromatic colors

Tropical plants such as *Phoenix* sp., *Fatshedera* sp. make you feel the summer warmth in the place where they are used, and hot climate plants such as cactus psychologically evoke the feeling of being in a warm place. A tall form plant like a tree in front of a narrow window gives a feeling of insecurity.

Along with plants, objects such as water, stones, and birds, fish that create psychological effects on humans either with their sound or movement should also be used indoors.

However, the emotions desired to be created by the designer may not have the same effect in every individual. In general, the features of the plant such as appearance and smell affect individuals, albeit differently. In this case, the plant determines the quality of the living space. Creating the desired effect in the space by using plants depends on the design type, but is also closely related to the young and mature appearance of the plant. While some plants have different leaf structure in their youth period, they may look different during maturity. For example, during the youth of *Monstera deliciosa*, the leaves are not very wide, the cleavage is very little and there are no holes on the leaves. In maturity, the leaves are splitted. Therefore, it should not be forgotten that the design is in a continuity and it should be planned that the effect created at the beginning of the design will change with the maturation of the plant. If the effect of the plant in the mature period will be used predominantly, mature plants should be preferred. Along with plants, another factor that guides the design is consumer behavior and habits. This situation gains importance especially in public places. Human behavior is also a determining factor for the organization of space created with plants in places such as waiting rooms, shopping areas, hospitals, terminals, stations that are exposed to intense human pressure. Inadequate and incorrect positioning of living area cannot be percieved by the user, or if it is insufficient number, it may cause usage errors.

RESULTS AND DISCUSSION

When living and planting solutions are organized together, a difference between functions can be seen in the space and aesthetic value will be added to the place via plants. However, human behavior and habits must be taken into account. Including plants is not enough to increase the aesthetic value of the place. The aesthetics and functionality of the space should be considered together with the seating units that will meet the desires of the individuals such as sitting, resting and watching. As can be seen in the figure below, in condition of lackage in sufficient number and properly designed seating spaces, the user will sit near the flower boxes. Thus, one will be exposed to physical contact that may adversely affect the plant

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CHAPTER 3

POT GERBERA PRODUCTION

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INTRODUCTION

Gerbera (*Gerbera jamesonii* L.), which is also known as Transvaal Daisy, African Daisy, Hilton and Barberton (Pattanashetti et al., 2012), spreads from Africa to Madagascar (Khosa et al., 2011) into tropical Asia and to South America (Tjia & Joiner, 1984; Bremer, 1994). It is a perennial herbaceous plant of the family Asteraceae and contains ±30 species (Hind, 2007). Gerbera was scientifically first described by J. D. Hooker in Curtis' botanical journal in 1889, as an African species, *Gerbera jamesonii* (Penningsfeld & Forchthammer, 1980).

Gerbera flowers display a variety of colours ranging from yellow and orange to red, pink, and white (Hansen, 1985) (Figure 1). The centre of the flower could be green or brown (Figure 2). The size of the flower is 7 to 12 centimetres and in the forms of single, semi-double and double (Figure 3).

The knowledge and experiences gained in the production of potted gerbera for many years in Bayindir, Izmir (Turkey) district under the Mediterranean climate conditions have been shared here.

Gerbera is a significantly commercial plant. Although its annual market value changes, it is always in high demand in the international flower market. The plant could be offered either as a pot plant (Figure 4) or as a cut flower (Figure 5 and Figure 6). It has fifth place in the cut flower market in the world after the rose, carnation, chrysanthemum, and tulip (Chauhan, 2005).

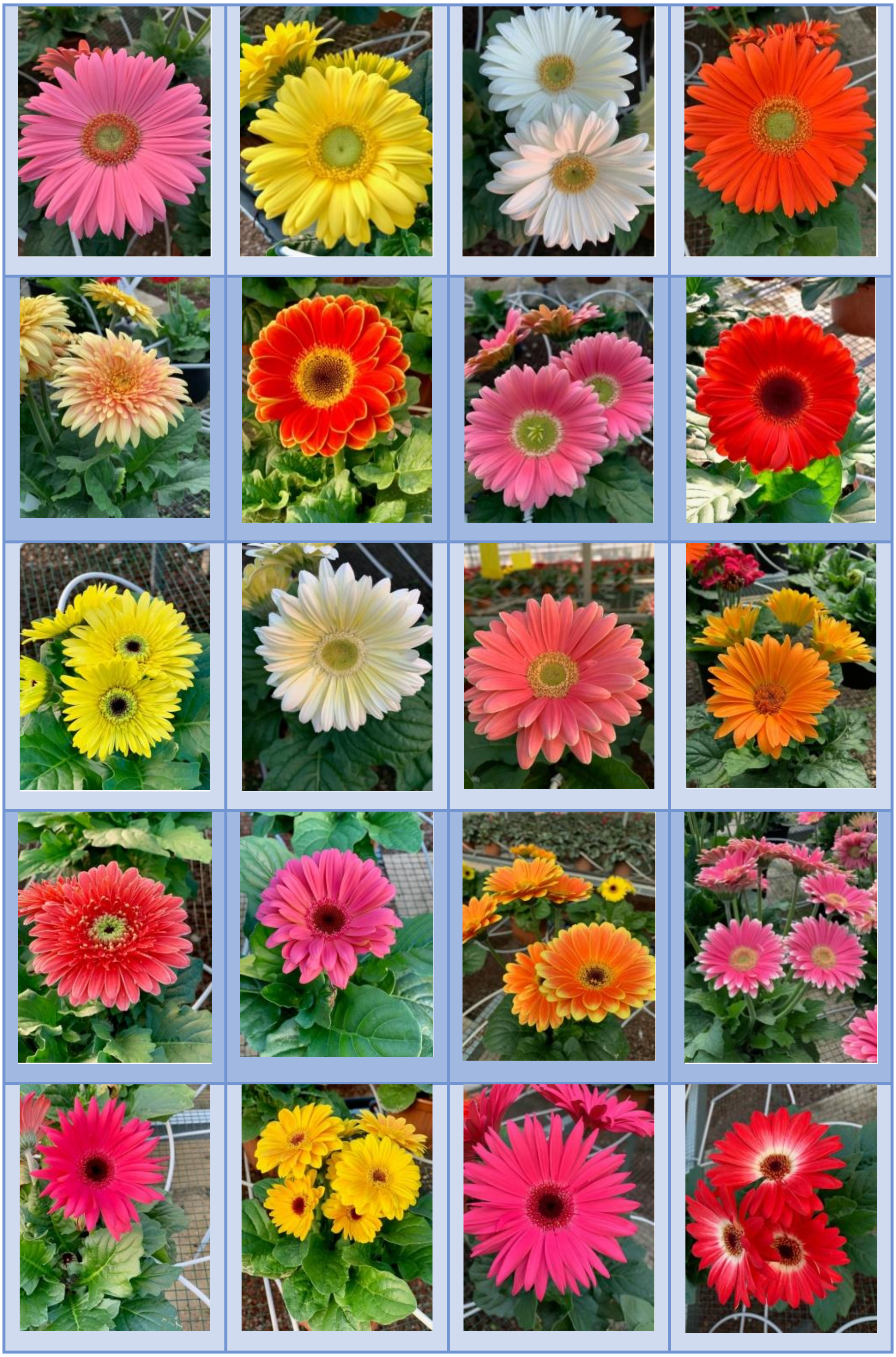

Figure 1: The Color range of gerbera (Original by Salman)

Figure 2: The green and crown centres of gerbera flower (Original by Salman)

Figure 3: The single, semi-double and double forms of flowers (Original by Salman)

Figure 4: An Example of pot gerbera production (Original by Salman)

Figure 5: An example of cut flower gerbera production (Original by Salman)

Most of the hybrids were obtained through the cross-pollination of *Gerbera jamesonii* and *Gerbera viridifolia*, both South African species (Leffring, 1973).

Figure 6: Gerbera as a cut flower (Original by Salman)

The variety of colours makes this flowering plant attractive for use in garden decorations, pots and cut flowers as it has a long vase life (Bose et al., 2003; Chung et al., 2005; Chauhan, 2005) (Figure 6). Gerbera, although it is a perennial plant, is treated as an annual. Accordingly, gerberas used as cut flowers are raised all year round; yet if they are used for pots, rising in winter is excluded.

Gerberas have been propagated through using seeds and cuttings or division of clumps and tissue culture (Leffring, 1971; Osiecki, 1988; Son et al., 2011). Seed propagation is rather simple. The plant has got larger seeds (250 pcs/g) in comparison to many of the seasonal bedding plants (Figure 7). Fresh seeds should be used which are obtained from a reliable source. The male and female parts of the same flower mature at different times to prevent the self-fertilization. Artificial pollination is used to obtain seeds, the harvest is in 3 to 4 weeks. With there being no seed dormancy and a short vegetation period, the plant is suitable for breeding research.

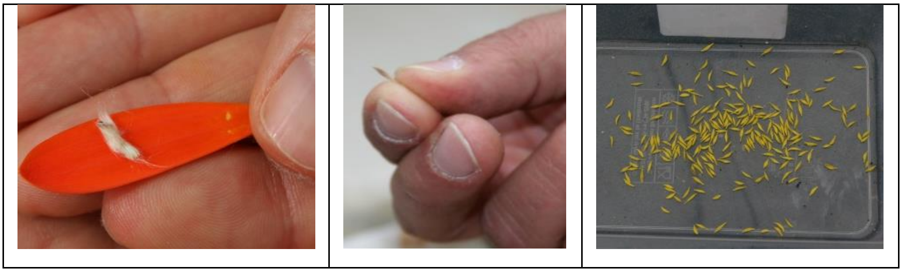

Figure 7: Seeds of gerbera (Original by Salman)

1. Seed Sowing

Seed propagation starts with sowing the seeds into seed trays that are filled with the sterile and well-drained peat (Figure 8). To increase plant growth in the germination stage, 20% perlite could be mixed with 80% peat. During this stage, the pH should be 5.5 - 5.8, EC 0.7 and the optimum temperature 22 - 24 ^{o}C.

Gerbera is a light-sensitive plant, therefore there is no need to cover the seeds after the sowing. Yet if the medium cannot be kept at 70 – 75% of humidity, the surface could be covered with a thin layer of vermiculite. If the germination is to be achieved in the darkroom, 12 hours of artificial lighting should be used. Germination, ideally, occurs in 4 – 5 days and cotyledons become visible (Figure 8). At this stage, seed trays should be taken to the greenhouses with a temperature of 20 – 22 ^{o}C and fresh air circulation. When the seedlings reach the 10th day, the humidity should be lowered to 20 – 30% and nitrogen from well-balanced calcium with 50 - 75 ppm should be applied.

Gerbera is sensitive to boron and iron deficiencies. A 0.25 ppm boron could be added during the fertilization process to support healthy seedling development. The growing medium should be left dry until the seedlings are just about to reach the wilting point. Overwatering and salinity adversely affect seedlings.

seed sowing	**germination**	**seedling**

Figure 8: Seeds, germination stage and seedlings of gerbera (Original by Salman)

When the seedlings reach the 14th day, EC level should be increased to 0.8 – 1.0 and, nitrogen level to 75 - 100 ppm. As the young leaves are sensitive to fertilizer salts they should be washed with fresh water. Using calcium nitrate together with 20-10-20 or 15-5-15 (N:K, 1:1) composite fertilizer in every second or third irrigation period increases growth health.

Young plants need an extra 3.200 – 5.400 lux up to 14 hours in darker climates. On the other hand, if the light is extensive, then the shading cloth (30 – 40%) is needed to reduce the stress and to prevent scorches.

Forty to fifty days after the sowing, seedlings should develop at least four leaves. This is the stage for potting which must be carried out before the root bounding occurs within the seed trays. Delay decreases the marketing time and causes premature flowering. At this stage, the environmental temperature should be kept at 16 ^oC.

2. Potting and Flowering

The potting medium should be sterile and well-drained with a level of 5.5 - 5.8 pH and 1.2 - 1.5 EC. One seedling should be used per pot and the plants must not be in touch with each other (Figure 9). Otherwise, leaves become over lagged, flowering becomes both delayed and decreased. The ideal temperature is 22 - 24 ^oC in daytime and 18 ^oC at night. The temperature should not fall under 15 ^oC to prevent delayed flowering.

Figure 9: Pot spacing of gerbera (Original by Salman)

Watering should be done through ebb and flow or drip irrigation and watering from above must be avoided. The uniformity of the humidity within the growing medium should be achieved and again, the medium must be left to dry until the next irrigation. At this stage, at first, a calcium nitrate fertilizer with 100 - 150 ppm N should be used and the level should be increased to 150 - 200 ppm. If the pH is over 6.0, chlorosis could occur due to iron and manganese deficiency. Also, every two weeks 15 - 30 ppm of magnesium sulphate ($MgSO_4$) should be added to avoid chlorosis around the main veins of the mature leaves. If the pH is over 6.2, iron deficiency may occur; the symptoms would be visible on the upper leaves. Boron deficiency shows itself as wrinkled dark green leaves. If the pH is under 5.5, manganese toxicity could happen, characterised with black blotches. It is advised to keep the pH between 5.5 – 5.8 and to add 0.25 ppm boron during usual fertilization. The ideal EC is 1.2 - 1.5.

Mature gerberas need high light intensity. If the daytime is shorter than 12 hours, 40 watts of artificial lighting should be used per square metre for up to 14 hours. During the short winter days, artificial lighting becomes a necessity for plant quality. The optimum light level for gerberas is 43.000 to 65.000 lux. Potted (10 – 15 cm) gerberas would flower in 13 – 18 weeks depending on the season and the level of the light intensity.

3. Growth Regulators

To control the stem height and to keep the perianth receptive to light 1.000 - 1.500 ppm Alar (B-9) should be applied 12 to 14 days after the repotting. This process could be repeated in two weeks if the need occurs. Depending on the pot size, additional growth regulator, PGR, can be added too. Spraying is to be avoided if the buds have reached pea size as it reduces the stem length and flower size (Figure 10).

Figure 10: The stage where the alar spraying should be avoided (Original by Salman)

Figure 11. Whitefly on gerbera (Original by Salman)

4. Pests and Diseases

The main pests include thrips, whiteflies, cyclamen mites and leaf miners. The dominant diseases are botrytis, phytopthora, powdery mildew, pythium and sclerotinia (Figure 11).

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CHAPTER 4

EDIBLE FLOWERS

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INTRODUCTION

Edible flowers were used as medicines and traditional dishes in ancient times. Nowadays, the acceptability depends on social group which shows the consumer's eating habit whether they cook them traditionally or for decoration of a meal, visual apperance with taste, their nutritive benefits, price and species of flowers.

Although there is no official list for edible flowers, Lu et al. (2015), reported 180 species of edible flowers belonging to 100 genera of 95 families. Artichoke, cauliflower and broccoli are some of the flower vegetables which are aceepted as vegetables. Some or whole parts of the flowers, belonging to ornamental plants, are edible like nasturtiums (*Trapaeloum majus*), chysanthemum (*Chrysanthemum* spp.), jasmine (*Jasminum* L.), hibiscus (*Hibiscus rosa-sinensis*), rose (*Rosa* spp.), pansy (*Viola x wittrockiana*). Likewise, some herb and fruit species have edible flowers like thyme (*Thymus vulgaris*), mint (*Mentha* spp.), sage (*Salvia officinalis*), dandelion *(Taraxacum officinale)* and cardoon *(Cynara cardunculus* L.), elderberry (*Sambucus* spp.), *Allium*s (chives, garlic,etc.) *Citrus* species (orange, lemon, etc.) (Chitrakar et al., 2019).

In this chapter, we'll deal with ornamental plants that have edible flowers and/or flower parts.

1. USAGE OF EDIBLE FLOWERS

Edible flowers can be used as dish decoration and garnish, like *Sambucus nigra* L. (Black elder), *Chyrysanthemum* spp.

(Chrysanthemum), *Jasminum* L. (Jasmine), *Tropaeolum* L. (Nasturtium), *Bellis* L. (Daisy), *Orchis* L. (Orchid), *Lavandula* L. (Lavander). *Rosa* L. (Roses), *Calendula officinalis* (Calendula flowers /marigold) are used in salads; *Lavandula* L. (Lavander) is used in sauces, *Borago officinalis* (Borage) , *Robinia pseudoaccacia* (Acacia), *Rosa* spp. (Roses) flowers and *Hibiscus sabdariffa* L. (Roselle) are used for aroma and flavor agents in salads, entrees, desserts and drinks; *Carthamus tinctoris* L. (Safflower), *Centaurea cyanus* L. (Bachelor's button), *Gardenia jasminoides* J. Ellis (*Gardenia capejasmine*), *Hibiscus rosa siriensis* L. (Chinese hibiscus), *Chrysanthemum morifolium* Ramat (Florist's daisy), *Jasminum sambac* L. (Sambac jasmine), *Rosa chinensis* Jacq., *Gomphrena globosa* L. (Amaranth flower) and *Lonicera japonica* Thunb (silver honeysuckle) are used as infusions or teas; *Capparis spinosa* L., *Hibiscus sabdariffa* L. (Roselle) and *Viola tricolor* L. (heartsease), are used in fermented drinks, vinegar and wine (Pires et al., 2019; Mlcek & Rop, 2011).

Before dealing with storage, processing, their nutritional quality, benefits and risks including safety tips, edible flowers should be identified clearly that includes scientific name which gives information about allergic and/or toxicity effects. They should be bought not from florists and garden centers because of pesticide and pathogen contamination. They should be grown and harvested as food or as food ingredient. Just after harvesting, it is the best way to cook edible flowers. Infact, flowers have high water content, they are perishable and this forces the sellers to use some additional methods to increase the shelf-life in fresh and/or dried form of flowers (Chitrakar et al., 2019).

2. STORAGE AND PROCESSING AFTER HARVEST

Harvesting of edible flowers should be in dry morning to maintain their flavor, smell and appearance. They should be kept in a plastic container in refrigeration conditions. Their shelf-life is between 2 and 5 days, that this short shelf-life limits their usage and commercilization. Therefore, it's obligatory to extend the shelf-life and preserve the flower quality like appearance, flavor and smell. The choice of preservation method depends on consumer's demand, selling price of the product, usage form, resources available, fixed costs (equipment, plant, etc.), and operational costs of the technology.

There are some methods used for preservation.

2.1. Drying

Drying is the simplest way if flower's shape is not very important. Sun drying, air drying, shade drying can be made. It has low production costs. Color and flavor of the flower retains around a year. The dried flowers can be used in teas, baked goods, candies, etc. After drying, cardboard boxes and airtight jars can be used for storage. Lavender, marigold and basil can be dried in these traditional methods.

The modern drying methods include, microwave drying (e.g. day-lily, eucommia, magnolia,liliflora) vacuum drying (e.g. rose petals) and hot-air drying (e.g. *Chrysanthemum boreale*).

Ahluwalia at al. (2014) studied with *Tagetes erecta* flower in 4 drying methods including, solar drying, cabinet drying, vacuum drying and fan drying methods. Vacuum drying method was determined as the best quality method for flowers, cabinet drying was the most economic. In all drying methods, flowers were higher in protein, ash, fiber and β-carotene content, but they were lower in total phenol content and antioxidant activity.

During the selection of drying method, temperature, time of applying, equipment used and cost of eqipment are very important by the deal of the usage of flower as it can be used for nutritive properties or as an ingredient or decoration.

2.2. Crystallization

Flowers are crystallized with sugar so that candied flowers can be used in cakes, desserts, candies like roses and violets. Crystallized flowers can be stored in sealed tin and plastic boxes (Nicolau & Gostin, 2016).

2.3. Preservation in Solutions

Vinegar, alcohol, honey and sugar syrups can be used as solutions. Because of osmotic pressure of the cells of flowers, the flavor and aroma gets in the solution and there becomes a floral vinegar as dresssing, a floral drink or cocktail, flower syrup that can be used in cakes and ice-creams, etc. Nasturtiums and roses can be preserved in solutions (Nicolau & Gostin, 2016).

2.4. Cold Preservation

Cold preservation includes refrigeration and freezing. To prolong shelf-life of flowers refrigeration is an important method. Bame (2004) studied with borage,viola, pansy, scarlet runner bean, and nasturtium flowers. In this study, refrigeration was applied in heat-sealed LDPE bags with holes (for oxygen and carbodioxide change) at temperatures between 20 °C to - 2°C. It was obtained that except borage all of the flowers, could be stored at 8°C for a week.

Freezing is also a method in cold preservation. When the frozen flowers are thawn, the colors become darker and textures are spoiled so that frozen flowers can be appropriate to use them in bakery, ice-cream, ice-cubes, iced-beverages (Nicolau & Gostin, 2016).

2.5. Modified Atmosphere Pacakaging (MAP)

The atmosphere inside the package is changed as increasing CO_2, reducing O_2 to decrease the metabollic activity and water loss and increase shelf-life. It can be applied to lily, rose and carnation.

MAP can be modified with gas mixtures of O_2, CO_2 and N_2 with different packaging materials like, PP, PE, etc. according to the studies with these combinations, it was observed that cauliflower's shelf-life extended to 30 days in cauliflower and with artificial neural networks extended to 50 days (Khaled et al., 2019, Aros et al., 2017).

2.6. High Hydrostatic Pressure (HHP)

In HHP method, pressure is used to 1000 mPa and then cool the process in a short time to deactivate enzymes and microorganisms. It is mostly used in food sector for nutritional and perishable foods. Because of high pressurized devices are used, it costs high. Fernandes et al. (2017) investigated it on centaurea, pansies, camelia and borage and in this study, the pressure level was 75 mPa for duration of 5 or 10 min gave the best result for pansies that extended the shelf-life from 6 days to 20 days. It revealed that HHP response depends on the type of flower.

2.7. Edible Coating Technology

Functional compounds like antioxidants, flavoring substances and nutrients are used as edible coatings mostly as a carrier. In addition they behave as barriers for color and flavors. It has some applications in edible flowers like Night-Fragrant flower that with the coating soy protein, sodium alginate and chitosan. In this study, it was reported that inhibition of shedding rate, weight loss, polyphenol oxidase inhibition and loss of Vitamin C could be assesed and its sensory quality improved (Xie, 2015).

Fernandes et al. (2018) studied with pansy flower with alginate coating. In this study, anthocyanins, antioxidants, hydrolysable tannins and flavonoid contents were obtained higher, shelf-life increased to 14 days and microbial load decreased and visual appearance preserved in the coated pansies.

2.8. Irradiation Technology

Although that irradiation has limited applications in food according to the regulatory allowances in EU, it's authorised as safe in USA regulations, it has some trials in flowers. It is a low-costed method. In the studies of Koike et al. (2015a) and Villavicencio et al. (2018), electron-beam and gamma irradiation were applied to *Bauchinia variegata* and *Viola tricolor* flowers. They obtained that irradiation did not affect phenolic content and antioxidant activity much, while prolonging shelf-life of flowers.

2.9. Freeze Drying (Lyophilization)

Freeze drying involves freezing, sublimation and vacuum evaporation of unfrozen water. It's determined as the most effective way to preserve flowers. In this method, shape, texture and color of the flowers can be retained. They had more phenol content, caretonids, antioxidant activity, etc. than the fresh flowers and other drying methods, like in the studies of carnations, roses (Chen et al., 2000), daylily (Mao et al., 2006; Que et al., 2007), orchid, rose, carnation, chrysanthemum, jasmine (Visalakshi et al., 2015), *Tagetes erecta* L. (Sirithon et al., 2012) and loquat flower (Zheng et al., 2015).

Freeze-drying has high initial cost, but it has more advantages like quality, shelf-life, nutritional effects and storage costs that the dealer should take care.

2.10. Hybrid Drying

Microwave –vacuum drying, vacuum and microwave assisted infrared radiation pulse-spouted bed freeze drying, microwave freeze drying are some of the hybrid drying technologies (Chitrakar et al., 2019).

Siriamornpun et al. (2012) applied hot air-drying , freeze drying and the combination of far-infrared radiation with hot-air drying to *Tagetes erecta.* They obtained the highest retention of antioxidant activity, carotenoids and phenolic compounds with the combination method.

Bae et al. (2009) studied with chrysanthemi flos flowers with far-infrared radiation resulting energy saved in drying, and marigold flower with far-infrared radiation with hot air convection drying, to determine better performance of drying for antioxidant activity and luteonin content.

In the combination of these methods, energy efficiency and improving product quality are very important factors to choose the preservation method.

3. NUTRITONAL IMPORTANCE OF EDIBLE FLOWERS

Flower has 3 components which is important in human nutrition. It has pollen, nectar and petals and other parts of flower. Pollen is rich with, carbohydrates, proteins, aminoacids, saturated and unsaturated fats, caretonoids, flavonoids, etc. Nectar is rich with sugars, proteins, aminoacids, lipids, phenolic substances, organic acids and inorganic ions, etc. Petals and other parts of flowers include also these substances with minerals, vitamins and antioxidants, etc. (Mlcek & Rop, 2011).

The studies in the last years are centered on the antioxidant, phenolic compounds (including anthocyanins and flavonoids) and the non-flavonoids. For example; it was determined in flowers of begonias, roses and garden nasturtiums that they include high antioxidant content, chrysanthemum as high in luteolin 7-O-glucoside which is the one of the most important flavonoid; in daylily flowers, catechin, chlorogenic acid, rutin and quercetin of phenolic compounds; in marigold flowers phenolic compounds. They obtained phenolic compounds mostly correlate with antioxidant activity (Mlcek & Rop, 2011). Table 1 includes some studies about nutritional properties of some flowers.

Color is an important organoleptic property which is affected by carotenoids and anthocyanins much. Anthocyanins have been authorised as food colorants in the European Union by SCF (EU Scientific Committee for Food) and JECFA (Joint FAO/WHO Expert Committee on Food Additives) and flowers can be used as a source of food colorants. Anthocyanins amount is possitively correlated with total flavonoids (Mlcek & Rop, 2011; Friedman et al., 2010; Mato et al., 2000).

In case of, inhibition of synthesis of total flavonoids, anthocyanins amount decreases and causes light color in the flower. Flavonoids and anthocyanins synthesis pathways have some common treat like, blue and violet colors are determined by delphnidin-based anthocyanins.

Lack of enzymes such as flavanone 3-hydroxylase and dihydroflavonol- 4-reductase causes pale colored flowers, e.g. The lack of delphnidin with the absence of flavonoid 3,5-hydroxylase causes light colored roses (Mlcek & Rop, 2011; Katsumoto et al., 2007, Mato et al., 2001; Stich et al, 1992).

Table 1: Nutritional and mineral composition of some flowers with bioactive compounds

		Nutritional composition (g/100g dry weight)								Mineral composition mg/100g dry weight						
Genus	**Species**	**Moist.**	**TC**	**Fiber**	**Protein**	**Fat**	**Ash**	**En.**	**Bio. Com.**	**Ca**	**Fe**	**K**	**Mn**	**Na**	**Zn**	**References**
Agave	*A. salmiana*	87.4	62.1	12.7	16.4	2.8	5.8									Sotelo et al., 2007; Fernandes et al., 2017
Allium	*A. schoenoprasum*		50.0	6.1	15.4	3.5		243	Unsaturated fatty acids (e.g. linoleic and oleic)							Grzeszczuk et al., 2011
Aloe	*A. vera*	89.5	56.8	13.8	16.4	4.2	8.6									Sotelo et al., 2007
Antirrhinum	*A. majus*	87.4	-	-	3.8	-	-	-	Antioxidants anthocyanins	283	3.5	$2.27*10^3$	4.5	70	7.0	Rop et al., 2012; Benvenuti et al., 2016
Bauhinia	*B. variegata L.* var. *candida alba*		77.1		10.90	7.0	5.03	415	Flovanols (e.g. quercetin, kaempferol), flavones (luteonin),fatty acids(e.g. linoleic,oleic,etc) tocopherols and organic acids (e.g. citric, malic,etc)							Villavicencio et al., 2018
Begonia	*Begonia boliviensis, Begonia semperflorens*	85.8-97.4	-	-	2.0	-	-	-	Antioxidants, anthocyanins, flavonids.	246	1.9	$1.30*10^3$	3.1	66	3.2	Landi et al., 2018; Rop et al., 2012; Benvenuti et al., 2016
Calendula	*Calendula officinalis*	89.3	62.1-81.3	13.1	6.4-13.6	3.6-5.3	7.7	151	Carotenoids (e.g. lycopene), Phenolic acid (e.g. coumarin), flavonoids (e.g. rutin)	41	-	-	0.1	1	-	Khalid & Teixeria da Silva, 2012; Rigane et al., 2013; Pires et al., 2017; Nicolau.& Gostin, 2016
Chrysanthemum	*C. frutescens*	90.4	-	-	7.2	-	-	-		270	5.4	$2.74*10^3$	8.2	93	5.7	Rop et al., 2012
	C. parthenium	90.1	-	-	6.9	-	-	-		346	5.9	$3.65*10^3$	7.4	115	6.0	Rop et al., 2012
	C. coronarium								Sesquiterpene lactone, pyrethrosin derivatives, dicaffeoylquinic acid.							Dokuparthi & Manikanta, 2015
	C.x morifolium								Apigenin, luteolin 7-O- (6'O-malonyl)-glucoside							Sugawara &Igarishi, 2009

Table 1 (continues)

Dianthus	*D. caryophyllus*	88.5	-	-	6.0	-	-	-	Anthocyanins (e.g. cyanidin 3-O-glucoside),	426	8.5	$3.07*10^3$	6.5	99	6.2	Zheng et al., 2019; Rop et al., 2012
Erythrina	*E. caribaea*	88.5	42.4	17.7	27.4	1.5	10.1	-								Sotelo et al., 2007
Fuchsia	*F. x hybrida*	91.6	-	-	2.9	-	-	-	Antioxidants anthocyanins	286	9.7	$2.35*10^3$	5.0	150	13.7	Benvenuti et al., 2016, Rop et al., 2012
Impatiens	*I. walleriana*	85.3	-	-	3.1	-	-	-		275	4.9	$1.92*10^3$	4.1	64	5.9	Rop et al., 2012
Rosa	*R. micrantha* and some *Rosa* spp.	71.6	86.1-90.2	-	4.3-7.6	1.3-2.0	4.2	465	Anthocyanins (e.g. cyanidin), carotenoids, essential oils,, phenolic acids as antioxidants (e.g. gallic acid), flavonols (e.g.kaempferol, quercetin) plant acids							Zheng et al., 2019; Guimaraes et al., 2010; Hirulkar & Agrawal, 2010; Voon et al., 2012; Biolley et al., 1994; Kumar et al., 2009; Pires et al., 2017
	R. odorata	89.9	-	-	2.6	-	-	-		273	3.5	$1.95*10^3$	3.4	76	4.5	Rop et al., 2012
Tagates	*T. erecta*	83.4	85.2	55.4	7.9	1.9	4.8	117	Antioxidants and anthocyanins Flavonols (e.g. quercetin, rutin), carotenoids (e.g. zeaxanthin), tannins, saponins.							Benvenuti et al., 2016; Fernandes et al., 2017, Navarro-Gonzalez et al., 2015; Kaisoon et al., 2011; Sirimornpun et al., 2012; Wang et al., 2006
	T. patula	90.6	-	-	3.1	-	-	-	Phenolic compounds (flavonoids (e.g. patulitrin and patulitin) and phenolic acids) fatty acids, their methyl esters, carotenoids (e.g.lutein, β-carotene, α-carotene, zeaxanthin	370	9.3	$4.06*10^3$	8.4	122	14.2	Park et al.,2017; Yasukawa & Kasahara, 2013; Rop et al., 2012; Faizi et al., 2011
Tropaeolum	*T. majus*	88.7-90.6	48.1-66.9	29.7-42.2	4.2-18.6	3.1-3.6	5.9-7.3	88-109	Phenolic compounds (e.g., hydroxycinnamic acid derivatives,etc.), antioxidants (e.g. ascorbic acid), carotenoids (e.g. lutein)	299	5.7	$2.18*10^3$	5.2	78	8.0	Benvenuti et al., 2016; Fernandes et al., 2017; Rop et al., 2012; Garzon & Wrolstad, 2009; Mlcek & Rop , 2011

Table 1 (continues)

Viola	*Viola x wittrockiana*	87.2-90.6	64.5	9.3	6.7-16.8	5.0	4.4	197	Antioxidants and anthocyanins	30- 486	7.3	$3.96 * 10^3$	0.1-7.9	1-132	11.5	Benvenuti et al., 2016; Vieira, 2013; Rop et al., 2012
Yucca	*Y. filifera*	88.1	-	8.5	25.9	2.1	9.7	-								Sotelo et al., 2007
Crocus	*C. sativus*	89.7								139	16.0	$1.40*10^3$	-	10	-	Serrano-Diaz et al., 2012
Dianthus	*D. caryophyllus*	88.5								426	8.5	$3.07*10^3$	6.5	99	6.2	Rop et al., 2012
Eruca	*E. vesicaria*								Polyglycosilated flavonids							Cavaiuolo & Ferrante, 2014
Hemerocallis	*H. fulva*								Polyphenols (e.g.(+) catechin, kaempferol, etc.) Flavonols (e.g. quercetin), Caffeoylquinic acid derivatives, flavones, phenolic glycosides e.g. isorhamnetin, phenethyl, tryptophan derivative, adenosine, guanosine, Carotenoids (lutein, etc)							Pires et al.,2019; Koike et al., 2015b; Cichewicz & Nair, 2002; Griesbach & Batdorf, 1995
Hibiscus	*H. rosa-sinensis, H. syriacus*		0.9	0.0	2.7	0.0			Anthocyanins, vitamins (e.g. thiamine, riboflavin, niacin, ascorbic acid), Polyphenolic compounds (e.g rutin, ferulic acid, luteolin) and flavonoids							USDA, 2018; Salem, et al., 2014; Vlase et al., 2014; Sheth, F. & De, S., 2012
Lavandula	*L. angustifolia*								Flavonols (e.g. rutin,quercetin, kaempferol), luteolin, triterpenoids,							Zheng et al.,2019; Rabiei et al., 2014
Ocimum	*O. basilicum*								Phenolic compounds (e.g, syringic acid, rosmarinic acid, , vanilic acid, etc.) Essential oils (e.g. estragol, linalool acetate, eugenol, menthol, cyclo-hexanol, etc.)							Bilal et al.,(2012)
Syringa	*S. vulgaris*								Flavanol glycosides (e.g. kaempferol 3-O-rutinoside), lignans, phenylethanoid glycosides (e.g. acteoside),							Kurkin, 2003; Su et al., 2015

Table 1 (continues)

Hemerocallis	*H. fulva*								Polyphenols (e.g.(+) catechin, kaempferol, etc.) flavonols (e.g. quercetin), caffeoylquinic acid derivatives, flavones, phenolic glycosides e.g. isorhamnetin, phenethyl, tryptophan derivative, adenosine, guanosine, carotenoids (lutein, etc)							Pires et al., 2019; Koike et al., 2015b; Cichewicz & Nair 2002; Griesbach & Batdorf, 1995
Hibiscus	*H. rosa-sinensis, H. syriacus*		0.9	0.0	2.7	0.0			Anthocyanins, vitamins (e.g. thiamine, riboflavin, niacin, ascorbic acid), polyphenolic compounds (e.g rutin, ferulic acid, luteolin) and flavonoids							USDA, 2018; Salem, et al., 2014; Vlase et al., 2014; Sheth & De, 2012
Lavandula	*L. angustifolia*								Flavonols (e.g. rutin,quercetin, kaempferol), luteolin, triterpenoids,							Zheng et al., 2019; Rabiei et al., 2014
Ocimum	*O. basilicum*								Phenolic compounds (e.g, syringic acid, rosmarinic acid, , vanilic acid, etc.) Essential oils (e.g. estragol, linalool acetate, eugenol, menthol, cyclo-hexanol, etc.)							Bilal et al., (2012)
Syringa	*S. vulgaris*								Flavanol glycosides (e.g. kaempferol 3-O-rutinoside), lignans, phenylethanoid glycosides (e.g. acteoside)							Kurkin, 2003; Su et al., 2015

4. SAFETY OF EDIBLE FLOWERS

Identification of edible flowers correctly is very important and this is first step of safety. E.g. dayliles can be eaten while the lily of the valley, belladonna lily and gloriosa lily are poisonous. The pickers should take care like mushroom pickers and use instructions from illustrated books.

Safety issues (between the year 2004 and 2018) for some edible flowers were reported by the European Union Rapid Alert System for Food and Feed (RASFF) They were, the presence of bacteria *Salmonella* spp., insectiside, insect repellent and sulfites. This report showed that edible flowers should be paid more attention in growing, preserving, storage, transfer and culinary conditions. There is no legal regulation for edible flowers marketing determined by WHO (World Health Organization), FDA (Food and Drug Administration), FAO (Food and Agriculture Organization of United Nations), and EFSA (European Food Safety Authority) that includes the list of flowers that can be eaten or used as a food ingredient/supply, growing and storage conditions with the use of substances.

The presence of some compounds like hydrogencyanide, erucic acid, coumarin, thujone is risky because of toxicity, so that they should be determined in Tolerable Daily Intake (TDI) or Acceptable Daily Intake (ADI) by JECFA or EFSA. Egebjerg et al. (2018) determined that consumption of 39.5 g *Tropaeolum majus* fresh flowers would exceed TDI in erucic acid and *Achillea millefolium* would exceed ADI of thujone. It was reported that, if these plants were used as decoration in foods, like cakes and desserts, doses were not exceeded but it should be paid more attention and controlled legally when they are used for bioactive substances as supplemantary foods and food additives (Pires et al., 2019; Egebjerg et al, 2018).

CONCLUSION

Flower is one of the basic factor of generative reproduction.to maintain the generation and to increase the variation in genetics. It has a natural defense mechanism that plant will protect its flower, while protecting, it can have some toxic materials. In addition, edible flower is high nutritive with protein, fiber, carbohydrates, minerals and bioactive compounds while lack of fats. Because of their genesis of high nutrition and probability of toxic effects, they should be consumed carefully and must be legally authorized as food additives, supplemantary foods and dietetic foods.

In the last days of popularity increased, edible flowers' shelf-life is one of the restricted factor in commercilization. More studies about preservation methods should be done to supply alternatives for different types of flowers.

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CHAPTER 5

DECORATIVE VEGETABLE: BOTTLE GOURD

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INTRODUCTION

For the physical and psychological comfort of people, plant growers have used their knowledge, experience and skills they have acquired over 10,000 years in food production and fin improving the environment. This type of plant cultivation has been a source of inspiration, especially for those who are just starting out in horticulture and making a career in this industry (Dixon, 2019). The idea and history of the design and presentation of decorative vegetable gardens goes back to Persia and ancient Egypt. Abbey and Versailles gardens and Roch-Guzaon etc. castle gardens are good examples in this regard. These have had a positive impact on their age. Medieval monastic gardens created a sector for the cultivation of fragrant vegetable plants. Residential and botanical gardens, affected by this theme, have occurred. Owning, watching and being informed about these plants have been a source of envy, both because of their appetizing nature, attractive flowers and the extra healing effects of some species. The inclusion of vegetables that can be used in nutrition, either as decorative plants or in combination with decorative plants, falls under the definition of edible decorative plant. Landscape possibilities from edible vegetables vary depending on the aesthetic and background purpose of the landscaper. These types of gardens are easy to access and maintain. However, vegetable types with special decorative features are effective in preventing monotony due to symmetry concerns (Aşur et al., 2010; Mihaela & Berar, 2013; Sarıçam & Çömlekçioğlu, 2013). Vegetable types should be kept rich in the establishment of decorative vegetable gardens (Larkom, 2002). It should be inevitable of recreational gardens established within the framework of landscape architecture principles, to be established from decorative plants or vegetables with ornamental-decorative features. In such areas, even a flower or a vegetable can be a decorative object if placed in appropriate spots. Everyone knows that vegetables are indispensable and healthy food products in daily life.

The term "edible landscape" is derived from the use of vegetables for decoration. It is possible to see vegetables which roots or tubers are used as a nutritional food, as the covering of the soil due to their magnificent leaf structures, cabbage in determining the borders, and edible vegetables with capsules or pods in the tower or pergola decorations. Green vegetables can be used to create image or color contrast. For example, growing parsley with straight and curly leaves together. There are very few vegetables that can be used for decorative purposes. With the correct placement, wonderful gardens can be created, even with vegetables in sight (Alp et al., 2010; Apahidean, 2011; Balcău & Apahidean, 2011).

1. USE OF GOURDS AS DECORATIVE PLANTS

With their interesting fruit shapes and colors, cucurbits are among the vegetables with very high decorative properties. Vegetables have a lot of reasons for their use in landscaping due to their natural characteristics (flower and leaf color, fruit and leaf shape, etc.) (Apahidean, 2011; Balcău & Apahidean, 2011). Bottle gourds are one of the most beautiful examples of these plants, especially in terms of its wrapping, spreading plant structure, fruit content, fruit structure and fruit shape.

Gourd is among the most interesting species of the plant kingdom in terms of fruit shape and cultivation purpose (Mladenović et al., 2013). It differs clearly from other cucurbites species with its different seed and fruit shape, white flowers and, of course,

characteristic fruits (Cutler & Whitaker, 1967; Akar, 2004). There are genotypes with rough fruit surfaces as well as gourd genotypes with smooth fruit surfaces. The roughness of fruit surfaces is controlled by a dominant single gene (Zhang et al., 2010). Taxonomic classification of gourds was made based on the morphological features determined by Heiser (1973). It has a diploid chromosome (2n = 22) structure (Wu et al., 2017). Male and female flower numbers and flower structure variability are the most important problems in cucurbits. The high number of male flowers in cucurbits, which generally have a monoic flower structure, and their availability at different times are the main problems. Despite the old genotypes, this problem is less in new varieties. In cucurbits which new varieties are andromonoic, as in the old genotypes, the male flower always exists before and in larger numbers than the female flower. The active ingredient in all cucurbits is cucurbitasin, and cucurbitasin is present in gourds both in a stressful and a stress-free environment (Mashilo et al., 2018). As a toxic metabolite, cucurbitasin, is toxic to many organisms. Therefore, it is considered as an effective weapon used by plants against pathogens and pests (Davidovich-Rikanati et al., 2015; Dube & Mashela, 2016; Shadung & Mashela, 2016). It is advocated by traditional healers to control diabetes, hypertension, liver diseases, weight loss and other related benefits. The Cucurbitaceae family of which gourd is also a member contains toxic tetracyclic triterpenoid compounds called cucurbitacins that cause bitter taste. There is no known antidote for this toxicity (Sharma et al., 2012; Ho et al., 2014).

A member of the Cucurbitaceae family, gourd can be used as a medicine in traditional medicine, as well as for decoration, accessories, musical instruments and household appliances, and has a rich content of unripe delicious fruits used in cooking as vegetables. Its usage area is quite high in tropical and temperate climates (Heiser, 1985; Robinson & Decker-Walters, 1997; Morimoto & Mvere, 2004; Shah et al., 2010; Mladenovıć et al., 2011; Mashilo et al., 2017; Wang et al., 2018). Even its fresh fruits are used in cooking as well as in making desserts and pickles (Sharma & Sengupta, 2013).

Too much speculation has been done on its origin. There are those who claim that it was raised by the natives before the discovery of America, as well as those who stated that it was brought to America from the Asian continent. However, it has been determined that gourds are originally of African origin (Erickson et al., 2005; Fuller et al., 2010; Kistler et al., 2014). Although the wild gourds found in Zimbabwe in their study by Decker-Walters et al. (2004), there are quite harsh discussions about the origin of the gourd plant (Erickson et al., 2005; Fuller et al., 2010). It has been determined that the oldest gourd residue found in the American continent evolved into the radiocarbon age (7290 years ± 120) (Doran et al., 1990). According to Erickson et al. (2005), gourds have a 10,000-year history in the Americas. According to archaeobotanical data in the Asian continent, cultured gourd remains were found in early Holocene settlements (Fuller et al., 2010). Stating that there is not a single fruit that can even come to the closest coast of the American continent by swimming the oceans without spoiling its seeds, Erickson et al. (2005) made the harshest exit about the origin of bottle gourd. Kistler et al. (2014), who studied the genomes of gourd, had the last word in this discussion. Although it differs morphologically with African gourds (Erickson et al., 2005), it has been determined that the origin of gourds in America is African (Whitaker & Carter, 1954; Decker-Walters, 1999; Doran et al., 1990). The oldest remains of gourds in the European continent are the remains of the Iron Age and they were found in Italy (Rottoli & Pessina, 2007). Although there is very little evidence in medieval Europe, it is understood from fruit and seed remains in Roman imperial areas that gourds were widely used in the Roman Empire (Schlumbaum &Vandorpe, 2012).

The difficulty of research in areas that were later covered with water does not answer the questions in Europe, America, Africa or even Asia, but also creates new questions. Gourds are likely to originate in Africa (south of the Sahara Desert) because a new wild gourd population was discovered in Zimbabwe (Decker-Walters et al., 2004).

However, it has been reported that gourd, also called calabash, is one of the oldest plants cultivated by humans. It is widely cultivated all over the world, especially in East Asian countries (Kistler et al., 2014).

The importance of cucurbits for agriculture is known all over the world. The solution for *Fusarium* (*Physarium oxyporum* f. sp. *niveum*) disease, which narrows the growing area of the one of the members of this family - watermelon vegetable, that causes a dramatic decrease in its yield, and even negatively affects both the appearance and the eating quality of the watermelon fruit, is also given by one of its own family (Cucurbitaceae). In order to grow watermelons in areas with *Fusarium* disease, it is necessary to use watermelon seedlings grafted on gourd. While it seems possible to grow watermelons in the same area after 3-5 years at the earliest with various cultural and chemical measures, gourd has become a rootstock to watermelon by showing resistance to this disease and as a result it can be grown even in large and old areas again. Gourds, which are resistant to *Fusarium* disease, are rootstocks to other cucurbit species also under low temperature conditions they tolerate (Davis et al., 2008; King et al., 2008). In the study conducted on the element Vanadium (V), which negatively affects plant life, it was determined that the watermelon plant grafted on gourd gave more positive results. It has been determined that the V density in the leaves of grafted watermelon plants is less than the control plants, that is, the gourd rootstock increases the V stress tolerance of the watermelon plant (Nawaz et al., 2018). In spite of all this, plant diseases are one of the factors that restrict gourd cultivation (Mashilo et al., 2016). As with all cucurbits, it should be cultivated in with good drainage, loamy, if possible, high sand ratio and soils rich in organic matter. Gourds can be cultivated with both seeds and seedlings. Seeds or seedlings are sowed and planted with 2 m row spacing and 1-1.5 m intrarow spacing. The sowing depth of the seeds is approximately 2.5-3 cm. On gourd plants grown in the summer season, cultural maintenance activities such as irrigation, fertilization and, of course, hoeing are vital for the healthy growth and productivity of the plant. Using stakes / hedges / arbor is important for fruit quality. It is also possible to grow by spreading on the soil surface, but because the fruits are exposed in this system, deformations occur in their colors.

In terms of income, one of the important ways of earning for families, cities - and of course countries - is tourism. Gourd has also been effective in tourism. It has gained an important and solid place among

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tourist gifts. Those who live in the countryside or make a living with handicrafts have made a profit by creating various products from gourd, and they still continue doing so. The people of Anatolia took their place in the stage with the gourd, as if they were making it just to spite since ancient times, against those who created excellent arts from pumpkin. Are you saying it is a livelihood-based occupation as well as making natural products, revealing creativity based on needs, using healthy, useful products, and habits, or it is continuing the tradition and living the current situation?

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Whatever we say, gourds have a great place in the lives of Anatolians and other societies. It can be a rare ornament of our guest room as well as the worker's water bottle in the field.

One of the most important reasons for the use of culture gourds in both nutrition, decoration and daily life is that the gourd has a waterproof feature that does not exist in the wild ones. The most important feature of using it as a water container is that it keeps the liquid cool. This feature stems from the narrow part called the neck. It is caused by the fact that the air in the wide area rises rapidly to the narrow and high area and the air in the narrow area is cooler than the large area, causing the temperature to decrease by evaporating, thus causing the environment to remain cool.

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Due to its lightness and usefulness, ladles made of gourd have been used for years in boiling water transfer instead of wooden scoops that do not float in water and sink due to their copper, steel or thick nature. Because it is hollow, in some parts of Anatolia, it has been named as *susak*, which means hollow squash (Tekin & Cantürk, 2014). The saying "now bottle guard juice purifies you" originates from the washing of the dead with gourd bowls (Bulut, 2018; URL-1). According to the belief of the Anatolian people, water should be boiled with wood fire to wash the dead and the principle of using non-man-made material (bowls derived from gourd was used for this reason) while washing the corpse. Some of its advantages are that it is very light and does not get damaged quickly when dropped even when full of water, but has a more stable drop than plastic. Considering the negative effects of plastic on nature and our health, we can say that the first users are predictive. Even life buoys were made from unprocessed dried gourds.

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Its use in Anatolia dates back to very old times. During the Ottoman period, those who could not swim used the gourd as a life buoy by tying a few of them around their waist. The use of gourds hanging from the pole in shooting (arrow) practices is another striking example of gourds for educational purposes (Üçer, 2001).

Also in Anatolia, gourd is also named as long squash, grapevine. The reason why it is called the gourd is because the gourd plant grows fast, allowing it to rise above the pergola. It got this name because of the view created by the fruits hanging from the pergolas. Especially in the Şanlıurfa region, there are bottle guard dishes with lamb meat, sour guard, and bottle guard made with tomato juice instead of water, or guard made using tomato paste. In Çorum İskilip, which is also in Şanlıurfa region, they make the gourd dish made using butter (URL-2). There are, of course, many bottle gourd dishes that we cannot reach in Anatolia.

Fried bottle guard fruit is made not only in Anatolia but also in China. Gourd soup is on the menu of the Chinese people. Dried gourd is marinated in Japan as well as is a component of roll sushi. In the Philippines, bottle guard cooked with pork and noodles is served on rice. It is consumed with many seafood in Vietnam, which has gourd genotypes known as Opo squash. The peel of gourd fruit is used in the production of chutney. In India, it is consumed with vegetables and fish

with added curry, especially fried together with tomatoes and potatoes, and moreover, it is used in dessert making (by adding milk and sugar to the grated bottle guard fruit). Main ingredients of the Hindus *Anapakaya* meal include tamarind juice, lentils, shrimp, and gourd. The drink made by grinding the fried gourd seeds with a mixture of rice, cinnamon and allspice is called the *horchata* drink. In both Korean countries, fried sun-dried gourd fruit is consumed. In Burma, gourd fruits are consumed by frying and using them in pastry making, as well the young leaves of the plant are also on the menu with their fermented spicy fish sauce (URL-3).

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The gourd with white flowers is an annual plant grown in tropical climates (Schlumbaum & Vandorpe, 2012). It is also named as deep gourd, Calabash, bottle gourd, long-neck squash, Tasmania bean, white flower gourd. The name Calabash comes from the Hawaiian language and rather than gourd, it also means a *large serving plate* in the center of the large dining table, made of wood. Unripe fruits are used as vegetables. Ripened and dried fruits were used as a tool for many different purposes.

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The gourds that serve humanity as funnels have become the life buoy around the waist of people who cannot swim, and the buoy that prevents primitive rafts from sinking in the water. One of the most interesting uses is in Levman management. However, it is used as drinking glasses, tobacco pipes (South Africa - even a gourd pipe was used to mimic Sherlock Holmes' pipe for easy balancing purposes.), and in South America (Brazil, Paraguay, Argentina, Chile and Uruguay) fermented beverages (tea-caffeine or Yerba Mate) was used in the form of a chalice as a symbol of closeness-friendship. Perhaps i was used in the most interesting and creative in India. Gourds were used as resonators in many instruments in this country. *Pungi* is an instrument used by snake charmers especially in India and the mouth part of this instrument is made of gourd fruit. It has been used in making instruments (percussion instrument, traditional violin) in Hawaii and West Africa. In addition, in Hawaii, in South America, gourd has become a plate that everyone on the table can share as a sign of friendship, close to its intended use. Chinese traditions are very rich and impressive in other matters as well as in gourds. In Chinese medicine, gourds are used as a container for carrying liquids (medicine and liquor) as well as for carrying medicines. We see the effect of gourd fruit texture on music in the use of Chinese handicraftsmen as a shelter for crickets that they hide in gourd containers of various designs. In China, gourds have also been

Pungi. valentine.gr

a shelter for animals bred for fighting ability. We encounter similarities

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between the use of gourds in West African societies and their uses in Anatolia. Some of

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these are washing (draining) rice, using it as a water container. In West Africa, gourds also used as palm wine glasses and soap dishes, even small ones (because gourds have genotypes from 5 cm long to 2 meters long) are used as ink and gunpowder container and

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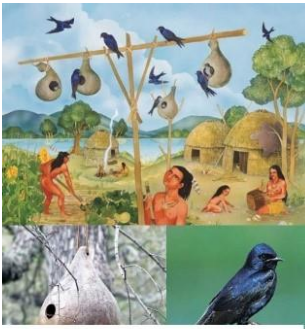
rattle (baby toy and women's necklace), it is even used as a bell. The long necked ones were recorded in flute making, large and big gourd fruits intented as pots and small ones as cups and glasses. In some parts of Africa, gourd has been used with the intention of hennaing the hands. It is stated that in the Philippines, gourds are even used as hats (URL-4). Again, due to its very natural structure, shepherds in remote settlements of Africa (Kenya and Tanzania Maasai people) used it both as a milking container and a milk flask.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Bottle gourds are one of the first examples of biological control in agriculture. The purple martins (*Progne subismor*) used by humans for biological control had a very special place for the Indians of Mexico and Native Americans. Because purple martins feed with insect which harm crops therefore they are beneficial to humans, people have built nests for these birds from bottle gourd in residential areas and their perimeters. The nests made of bottle gourd have also caused rapid increases in the swallow population due to their larger size and distance from their enemies. This plant is even sacred in some places: Kenya, Benin, Haiti, Jamaica, and Dominican Republic… It represents the rattle of the Vodou priests. Luos have relied on trumpets they gathered from gourds to keep unwanted and wild animals away in their sacred rituals. Again in the similar region -St. Lucia it is considered a national plant. Nowadays, bottle gourds, which are used only in decoration in developed and developing countries, have been among the basic instrumentals of religious and spiritual ceremonies (URL-4).


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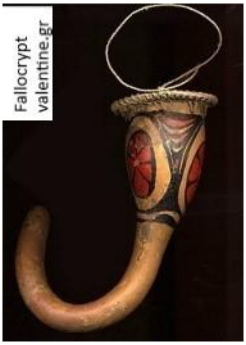
While the newly dried gourd, which has lost its freshness, is vibrant yellow in color, turns dark with time and use. However, the color is darkened from time to time, especially in the African region, by fumigated with smoke. Apart from these natural colors, they are painted in many different colors for color



preferences. However, we can say that no paint is applied to those who are used as a tool in nutrition and daily life, although they are designed. The only reason why gourd causes significant divergence among many anthropologists is that it is used as a sheath to cover the genitals of some ethnic group male members in New Guinea. Is the purpose of the use as a *Fallocrypt* (penis sheath) *only to protect* or *is it having other function*? Most anthropologists believe that the gourd's role here is not only protection but also *a valuable social function*. In the Inca civilization, the people depicted the very valuable folklore symbology on the gourd. In Hindu traditions, they benefited from dried gourds as pots, as gourd juice was believed to have healing properties. Making decorative items from gourd, and the use of these tools in the tourism sector over time, thus the beginning of becoming an economic figure dates back to the Native Americans and especially the people of Peru centuries ago. So the use of gourds in decoration is a very old tradition

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(URL-4). In addition to its natural shape, it is also used by shaping it with various bending and twisting movements while it is still fresh. In addition to these, it is also used by cutting, carving, cultivating in clay pots for patterning or by patterning later. For the purpose of using the tool, equipment or any equipment to be obtained from the gourd, sometimes the gourd fruit is harvested from the plant even when it is still the size of a walnut (rattle necklace purposes). However, it is expected that the gourd fruit is fully ripe on the plant when used for tools and equipment purposes and in its natural form.

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In addition to using it in its most natural form and without any processing, gourds are shaped by processing with electric wood burners and high-speed and pencil-like tools that provide the convenience of drawing all kinds of designs on woods, and is recruited to various tools and equipment, shelters, decorative items, or any vehicle with important functions and other things. The reason why gourds serve life in such a wide area is not only because they are easy to find, cheap and in high demand, but also because of their natural healthiness.

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From lady dancer figures to simple drinking glasses, various decorative or life-use materials are obtained from gourds. The gourds, which can be a bird's nest when a hole is sufficiently wide (wide enough for a bird to enter) from where the narrowing begins from the main navel, can be cut under the narrowed place above the navel and can also be used as bowl, pencil holder or food container. The fruit of those curved in the shape of a swan neck of the narrow part can kept upside down and it can take the form of a table lamp by cutting it with symmetry cuts and spaced apart the way that the wide part will be like a lamp shade. Narrow, long and flat ones are also known to be shaped like lampshades by cutting wide areas at appropriate intervals. It is known that the electrical system is laid in the fruit by drilling enough holes at the top of the narrow area to allow the electric cable to pass. When the narrow and long part of the curved-necked fruits, which fits the purpose, is cut from the center after slightly expanding, it can be used both as a pipo for decoration as well as direct purpose. The use of very large fruits (especially available in wild gourds) as dishes is common. Today's gourd fruits are known to have higher durability than their ancestors. By separating the wide parts from the narrow and long parts, it is used in large food bowls, rice washing-straining, separating various cereal products from stone and garbage, straws, even used as a hat (indigenous

tribes in Kenya) by turning it inside out. The fruits of large-fruited gourds are also used to knead the traditional mixture of butter and clay with each other or to make butter in Kenya and in places with similar lifestyles (URL-4).

The gourd fruit is often used as a *resonator* in the music industry. Especially resonators made of gourd fruits increase the quality of the musical tone by increasing the acoustic power and balancing the intensity of the harmonic tones relatively. Shaker is an important musical instrument created by taking advantage of the inner acoustics of gourd fruit. Shaker is one of the oldest known examples of musical instruments. The female flowers of the *Coix lacryma-jobi* (tear grass-Eyup's tears) plant with hollow female flowers hit the gourd belly, producing a high quality high churning sound. The curved neck of the gourd fruit is also used as a handle in this musical instrument (URL-4). Traditional violins of Africa called *goje* are made from gourds. The gourd fruit, which is even used in making puppets, has been a source of inspiration for the construction of stadiums. The gourd fruit was the inspiration for the Soccer City stadium built in Johannesburg, South Africa, which hosted the 2010 FIFA World Cup. The German poet Walahfrid Strabo (808-849), an adviser to the Carolingian kings, wrote that one of the 23 plants that should be kept in gardens to be established for pleasure should be gourd (URL-3).

CONCLUSION

There are very few plants that both serve humanity in nutrition and meet our daily needs. While we use only the fruits of most plants for nutritional purposes, some of them are used only in clothing and some in cosmetics. However, bottle gourd vegetable is used in nutrition, decoration, making daily used tools and equipment, as a hat or carrying medicines, and even chalice. Bottle gourds are also known to be used for fishing or fish transportation. If you can find plenty of it, it will serve humanity as fuel. Many fruits and vegetables or decorative plant fruits do not completely dry on the plant without rotting. Even if it dries, the fruit cannot maintain its shape without shriveling or wrinkling or being warped.

Bottle gourds is one of the rare plants-vegetables that can be used as traditional and advanced medicine (based on cucurbitacine content), accessories, landscaping, musical instruments, daily used tools and equipment, and even as canopy and hedge plants. In addition to its characteristics such as different flower color, very interesting fruit and seed shape, growing style, gourds are also known to be grown in landscaping and decorative gardens.

The wide variety of gourds is known to be used as an advantage due to cross pollination. There is a need to develop new varieties of this plant (especially hybrid) with breeding programs. The new developed varieties are likely to be even more productive in much different flower colors and shapes, different fruit shapes and patterns.

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CHAPTER 6

A STUDY ON PHENOLOGICAL CHARACTERISTICS OF *FICUS CARICA* AND *MORUS ALBA* ‘PENDULA’ USED IN GREEN AREAS IN ISKENDERUN AS A MEDITERRANEAN COASTAL CITY

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INTRODUCTION

Today, areas that are not exposed to construction are defined as green areas, as opposed to man-made systems such as buildings, roads, factories. Green areas have many elements that form green systems from small neighborhood parks to dense forest areas, from independent point areas to corridors and spots (Forman, 2008). The concept of green space can also be defined as open spaces. Keles (1970) stated that agricultural lands and natural areas that are outside of the development are open areas. However, open spaces are less open to construction or filling than green areas (Forman, 2008). Therefore, the content of open space and green space concepts can be diversified according to the perspective. In this study, the two concepts are evaluated in a similar sense.

The society of 55.7% in the world and 75.6% in urban areas live in Turkey because the green areas in urban and rural areas have become more important nowadays (The World Data Bank Report, 2019). The rapid increase in the built environment depending on the population has resulted in increasing the number of green areas, keeping the green network systems up-to-date, and developing new strategies (Guzel & Türer Başkaya, 2020). Green areas are used as a buffer zone near residential areas due to their visual and physical effects, as well as sports fields, parks, and social services for individuals (Ozturk & Bozdogan, 2014; James et al., 2015; Klompmaker et al., 2018). In addition, it has a protective function as it contributes to the treatment of many diseases and creates environments where individuals are happy

(D'Alessandro et al., 2015). An important part of the green areas is university campus areas. These areas have the characteristics of a small city. While campus areas create aesthetic, functional, and social areas for the units they contain; also it contributes to the urban green space system (Sogut et al., 2013; Korkmaz & Bozdogan, 2014). In this case, the university campus is a testament to the many functions that make living spaces. The campuses, which are generally located far from the city center, also interact with the surrounding rural areas. Therefore, these campuses should be handled in three parts (natural-semi-natural areas, education areas, and buffer lines) in terms of planting; It should be designed in a way to support, protect and sustain the natural structure. Campus plantings it is also important for the protection and promotion of wildlife (Sogut et al., 2013). For this reason, the use of ornamental fruit trees, which will contribute to wildlife as food, in campus areas is also considered an important strategy in terms of sustainability.

As in many regions of our country, fruit trees are used extensively in planting designs in the green areas of the Mediterranean Region (Durmus & Yigit, 2003; Yılmaz et al., 2018). The use of fruit trees in urban areas is a controversial issue in the landscape architecture profession. The reasons for this are thought to be that spilled fruits pollute the environment, attract insects and bees, and cause vandalism. However, social support is also provided by the use of edible trees in urban green areas. Green areas have many positive contributions to the urban ecosystem. These include the ability to reduce the urban heat island effect, absorb noise, regulate the humidity of the air, reduce/eliminate pollution, and regulate climatic events that affect urban living comfort (Aksoy, 2001). Cetin et al. (2020) determined that surfaces covered with plants, from trees to ground cover plants, protect individuals' comfort areas. Ozturk & Bozdogan (2015), on the other hand, stated that the green areas along the roads reduce traffic-related pollution and increase the air quality; Sogut et al. (2018) stated that such green areas create a buffer zone against toxic substances. Therefore, green areas have features that increase the quality of life in urban areas with their improvement and protection effect. Plant species selection is an important factor that increases the success of the design; it plays an active role in the social and economic integration of urban living spaces and design (Ekici, 2010; Guler et al., 2017). COST Action E12 survey (the program examining all plant species in Europe) reveals that the plant species should be determined following the field. But globalization, urban green areas in Turkey are increasing the use of exotic plant species. Not including native species in nurseries is one of the most important reasons for the increase in the use of exotic species (Baser & Yildizci, 2011; Kayabası, 2018). However, in terms of the sustainability of the urban landscape, the use of native species in landscape applications provides economic and ecological support to the country's resources. Bekci et al. (2013), by referring to the development of native species alternatives instead of exotic species used in planting designs in urban areas in the Bartın region, revealed the identity of our country and the adequacy of production resources.

The Mediterranean Region allows the growth of drought-resistant, leathery-leaved, evergreen trees and shrubs due to hot and dry summers and mild and rainy winters (Sayan, 2001). The cultural vegetation of the region consists of exotic plants and even some of these species can adapt to the region and become natural. Yılmaz et al. (2013) determined that exotic species are preferred over native species due to their aesthetic value and that they have adapted to the region in both ecological and cultural scales in green areas in Antalya, Guler et al. (2017), on the other hand, that *Arbutus unedo* L., the native species of the Mediterranean Region, can be used in landscape design applications with its aesthetic and ecological features; they reported that it provides many opportunities in terms of economy with its fruit.

In green areas, creating designs with high visual value by paying attention to the aesthetic and functional features of the plants can be the main goal. At this stage, the physiological characteristics of plants (flower, leaf, bud, fruit, branching), ecological demands, and time-dependent changes are taken into account (Bekci et al., 2013). Besides, climate change, which is a global problem, should not be ignored. Deviations from precipitation in Turkey, to decrease the precipitation is caused to reach the endpoint of seasonal temperature. In the Mediterranean Region, as the dry-mild seasons are arid, the moisture level of the soils decreases, and changes are observed in the leaf-flower-fruit formation of plants (Ozturk, 2002). Changes in microclimatic values also affect plant phenology. Morin et al. (2009), the continuity of high temperatures caused by climate change causes abnormal budburst, early foliation and frost damage, and late foliation due to late cooling in temperate zone plants. Doi & Katano (2008) examined the relationship between the growth of plants in Japan and climate change; He determined that the change in spring temperature values delayed leaf budding and the vegetation period was prolonged in the plant. Paltineanu & Chitu (2020) stated that the first stages in plant growth are directly affected by the maximum temperature; during the 50-year observation period of *Malus* and *Prunus* species, the growth of the buds (10-14 days), the first bud emergence (9-15 days), the beginning of flowering (7-11 days) and the end of the flowering (2-7 days) began to be seen in later dates. This situation causes changes in the visual activity period where the ornamental quality of the plant is at the forefront and the application times of some maintenance procedures.

The aim of the study is to examine the relationship between the changes in the phenological characteristics of the *Ficus carica* and *Morus alba* 'Pendula' and the climate data of the landscape designs in Iskenderun Technical University Central Campus, which is located in the Mediterranean coastal city of Iskenderun and has an area of approximately 800 decares. In terms of determining the visual activity duration, which is an important parameter of landscape designs.

MATERIAL AND METHOD

Material

The study was carried out in Iskenderun Technical University Central Campus, located in Iskenderun, the district of Hatay, which is on the Mediterranean coast between January 2019 and October 2020 **(Figure 1).** The climate data of the study area for the years 2019-2020 were obtained from Turkish State Meteorological Service (**Table 1).**

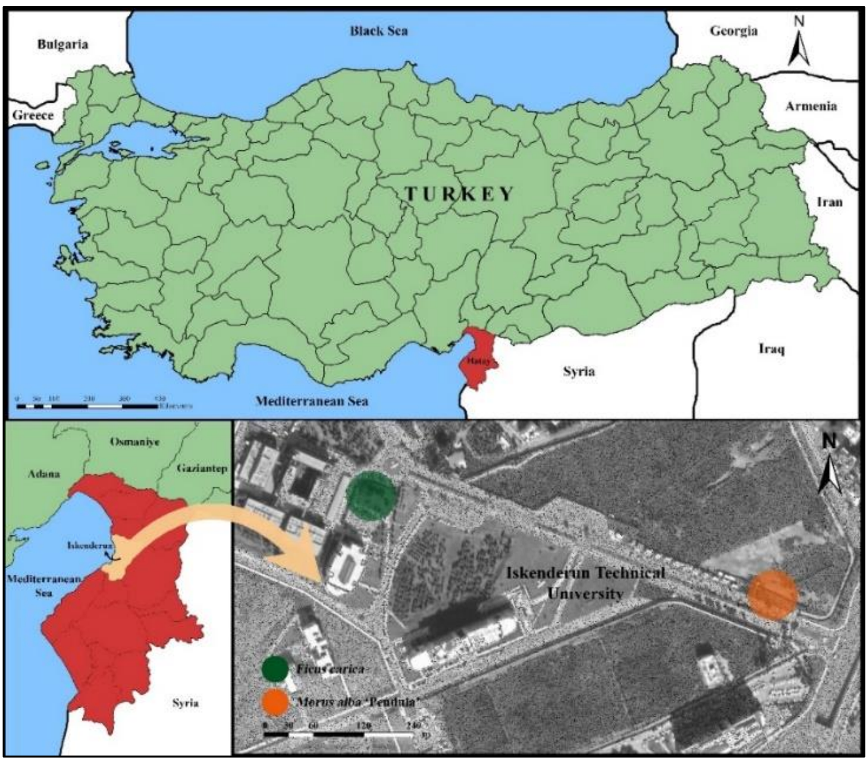

Figure 1: Study area

Table 1. Some climate data of Iskenderun city according to months of 2019-2020 (Turkish State Meteorological Service, 2019-2020)

Month	Monthly Average Temperature (⁰C)		Monthly Average Maximum Temperature (⁰C)		Monthly Average Minimum Temperature (⁰C)		Monthly Average Relative Humiditiy (%)		Monthly Total Precipitation (mm)	
	2019	2020	2019	2020	2019	2020	2019	2020	2019	2020
Jan.	12.4	12.0	15.7	15.6	9.63	9.2	57.4	59.4	167.0	65.8
Feb.	13.9	12.3	17.7	15.6	10.6	9.5	62.2	55.0	145.5	66.8
Mar.	15.2	16.6	18.8	20.5	12.0	13.2	65.6	66.3	108.0	180.6
Ap.	18.0	18.7	22.3	22.1	14.4	15.7	64.6	68.5	84.8	73.6
May	23.7	23.4	28.1	27.8	20.4	19.9	62.1	60.9	3.8	60.1
June	26.9	25.1	29.9	28.4	24.3	22.5	70.3	69.9	43.2	30.4
July	28.5	29.0	31.4	31.8	25.8	26.8	67.3	74.5	85.0	0.2
Aug.	29.6	29.8	32.5	33.1	27.2	27.2	68.2	66.0	6.6	17.4
Sep.	28.0	29.7	31.5	33.4	24.8	26.9	62.2	66.2	31.4	11.6
Oct.	25.5	27.0	29.5	30.9	22.2	23.7	58.5	53.4	35.2	0.0
Nov.	20.4	-	24.8	-	16.9		46.4	-	63.8	-
Dec.	14.3	-	17.8	-	11.6		66.9	-	164.6	-

Plant Material

In the study, *Ficus carica* and *Morus alba* 'Pendula' species, which have been adapted to the regional climate, are preferred due to their color and form features in landscape architecture studies and are used extensively in the study area (**Figure 2).**

Ficus carica: This species, whose Turkish is fig, is distributed in many regions such as America, South-Southwest Africa, Australia, and Europe with the subtropical climate of the world. Soil moisture requirement is 40-45%, annual rainfall requirement is 500-550 mm, and growing temperature requirement is 20 °C. The species, which has been used socially and economically from the past to the present, is also preferred as an ornamental plant. Fig, which is considered native for our country, is a tree with a sparsely branched, sparse crown, growing 8-10 meters long, shedding leaves in winter, growing in the form of a single or multi-stem or hearth. Old branches are ashen and fresh shoots are olive green. Its body is gray-brown. The leaves are hand-shaped, with 3-7 lobes, the upper face is dark green-rough, the lower surfaces are light green-hairy. Leaf length is 8-20 cm and width is 6-18 cm. Its leaves turn yellow in autumn. The fig flowers are lined up in the receptacle, which is called the fruit, as of the moment they first appear. It is used as a solitary in landscape applications (Ozbek, 1978; Polat & Calıskan, 2008, Gul & Ozrenk, 2019).

***Morus alba* 'Pendula'**: It is a cultivated plant produced by grafting. It is a small shrub with a thick leaf with weeping form that can grow 4-6 m. Although it's native in northern China, it naturalized in nearly every region of Turkey. Soil moisture requirement is 65-80%, annual rainfall requirement is 600-2500 mm, and growing temperature requirement is 24-28 °C. Trunk bark is light-gray-brown colored and cracked. Its leaves are heart-shaped, 6-12 cm long, 3 deep lobes, upper face is bright green, lower face is matt. Leaf edges are blunt and coarse toothed, the petiole is 1-2 cm. In autumn, leaves turn bright yellow / brown color. The flowers that bloom in April-May are in a hanging form. Male flowers 1-3 cm; light yellow boards, female flowers form light green boards 3-5 mm in length. Female flowers turn into the compound, white or pinkish colored fruits in June-July. Fruits are 1-3 cm in length and 6-20 mm in width; it has 5-10 mm fruit stem. It grows well in temperate climates, sunny places, and deep soils. It is resistant to salty, calcareous, sandy soils, and cold weather conditions. It is a highly adaptable plant. The visual value of the species is quite high depending on the habitus. Therefore, it is used in parks and gardens as a solitary or as a group plant for orientation (Pamay, 1992; Mamıkoglu, 2007; Gunduz et al., 2009, Gunes, 2013).

Figure 2: Species Selected in the Scope of Study (Original by Sert)

Method

The study was carried out in three stages. In the first stage, literature research has been done on urban green spaces and the importance of species selection in planting design and changes in plant phenology depending on climate. In the second stage, the phenological characteristics of *Morus alba* 'Pendula' and *Ficus carica* species, which are members of the Moraceae family, are observed in the green areas of Iskenderun Technical University Central Campus. At this stage, 3 individuals from both species were selected in the campus area; in the period of January 2019-October 2020, the data were processed into the observation form every week for 22 months and a photo was taken. Within the scope of these observations, data on the following parameters were obtained.

- The starting of leaf budburst
- Leaf unfolding
- Blooming
- Under ripe fruit
- Mid-ripe fruit
- Ripe fruit
- Ending fruit
- The beginning of leaf discoloration
- The starting of leaf fall

In the last stage, the effects of the arid-semi-arid climate characteristics of the region on the phenological characteristics of the plant species were evaluated periodically by comparing the observation data obtained with the microclimatic data (average temperature, maximum temperature, minimum temperature, total precipitation, average relative humidity) obtained from the İskenderun Meteorology Directorate. Suggestions on the subject are presented in the example of Iskenderun city. The results of the study are planned to be used for other cities with coasts to the Mediterranean and to guide vegetative designs according to the changes in the visual activity periods of plants in landscape architecture studies.

RESULTS

In the study, *Ficus carica* and *Morus alba* 'Pendula' species, which are used extensively in the green areas of Iskenderun Technical University campus and are members of the Moraceae family, were monitored for 22 months between January 2019 and October 2020.

Table 2: The form of phenological observation for Iskenderun Technical University Central Campus plants

Phenological Observation Parameter	***Ficus carica***		***Morus alba* 'Pendula'**	
	2019	**2020**	**2019**	**2020**
The starting of leaf budburst	8 February	14 February	22 February	14 February
Leaf unfolding	8 March	14 March	22 March	14 March
Blooming	8 March	14 March	22 March	20 March
Underripe fruit	15 March	27 March	5 April	27 March
Mid-ripe fruit	12 July	12 June	19 April	27 May
Ripe fruit	16 August	17 July	3 May	19 June
Ending fruit	11 October	30 October	21 June	24 July
The beginning of leaf discoloration	20 September	4 September	20 September	28 August
The starting of leaf fall	18 October	18 September	18 October	4 September

Accordingly, the process between the leaf bud formation, when the vegetation period begins, and the complete fall of the leaves, when the vegetation period ends, is shown in Figure 3 monthly. The results obtained are given in Table 2; evaluations were made based on species.

Evaluation in Terms of *Ficus carica*: When the observation data of *Ficus carica* obtained in a period of 22 months are examined; the appearance of the first leaf budburst in 2019 on February 8; in 2020, it took place on February 14, 6 days later. The first leaf formation (leaf unfolding) and the beginning of flowering for the species were observed on 8 March in 2019, and again on 14 March in 2020. This situation indicates that flowering and foliation occur at the same time. According to the data, there is a one-month period between the first leaf bud emergence and the leafing/flowering period. Young fruit formation after flowering was observed on 15 March in 2019 and on 27 March in 2020. According to this; one week in the first year from flowering period to fruit formation; in the second year, there was a two-week process. When the ripening period of the fruits is examined; in 2019, the transformation of young fruits into medium-ripe fruit was seen on July 12, after 117 days (4 months), and in 2020, on June 12, after 72 days (2.5 months). The fruit's transformation from mid-ripe to mature fruit on August 16 in 2019, on July 17 in 2020; It took place in approximately 34-35 days. The ripe fruit in *Ficus carica* was finished on October 11 in 2019 and on October 30 in 2020.

Figure 3: Phenological observation ımages of *Ficus carica* and *Morus alba* 'Pendula' species for the period 2019-2020

Yellowing of the leaves started on September 20 in 2019 and on September 4 in 2020, 16 days earlier than the previous year. The defoliation started on October 18 in 2019 and on September 18 in 2020. All these data obtained show that the phenological characteristics of *Ficus carica* are affected by the climate parameters. In this context, the monthly average temperature obtained in January and February in the same months of 2019 in 2020 is lower than the monthly maximum and minimum temperature values; It was determined that the first leaf bud formation, leaf formation, and flower formation were negatively affected by this situation. It is accepted that the duration of activity is high in terms of fruit in the process from the formation of young fruit in the plant to the end of the fruit. Accordingly, this process is shorter in 2020 than in 2019. When the climate data were evaluated in terms of this process, it was determined that the monthly average temperature, monthly average temperature, monthly maximum, and minimum temperature values were lower in 2020 especially for May and June. Also, when the monthly total precipitation amounts between the month of March when fruit formation takes place and October when the fruit ends; it is seen that there is approximately 1.8 times more rainfall in 2020 than in 2019. Similarly, climatic data affected the yellowing and shedding times of the leaves. Yellowing of leaves in 2020 is 16 days compared to 2019; leaf fall was also observed 30 days earlier. It is thought that this situation is affected by the total rainfall during the summer period and the monthly average, minimum and maximum temperature values. Accordingly, the total rainfall in July-August-

September in 2019 constitutes 60% of the year-round rainfall and 8% in 2020.

Evaluation in terms of *Morus alba* 'Pendula': When the 22-month vegetation period *of Morus alba* 'Pendula' species is evaluated; the first leaf bud appeared on February 22 in 2019, and on February 14, 8 days earlier in 2020. The first leaf formation was seen on March 22 in 2019 and on March 14 in 2020. According to the observation results, there is a one-month period between the first leaf bud and leaf formation. The flowering period was observed on 22 March in 2019 and 20 March in 2020. Accordingly, flower and leaf formation takes place on the same date. However, in 2020 flower formation took place 6 days later than leaf formation. Immediately after flowering, young fruit formation was observed on April 5 in 2019, and on March 27 in 2020. The transformation of the young fruit into medium-ripe fruit took place on April 19 in 2019 and on May 27 in 2020. Mature fruit formation was observed on May 3 in 2019 and on June 19 in 2020. If the ripening fruits are finished; it was seen on June 21 in 2019 and on July 24 in 2020. According to the results, young-medium mature-ripe fruit formation occurred at different times in both years. In 2019, there are 42 days from young fruit to mature fruit formation. Mature fruit formation continued for 49 days. In 2020, the period from young fruit to mature fruit was 91 days. Mature fruit formation continued for 35 days. This situation, that is, the increase in the ripening process of the fruit, can be considered important in terms of increasing the effective period of the fruit with the fruit. Yellowing of the leaves of the plant started on September 20 in 2019 and on August 28 in 2020. The defoliation period started on October 18 in 2019 and on September 4 in 2020. All the data obtained show that some phenological characteristics of *Morus alba* 'Pendula are affected by climatic parameters. In this context, firstly, leaf bud formation and first leaf emergence occurred 8 days earlier than the previous year, although the monthly average temperature, monthly maximum and minimum temperature values obtained in January and February 2020 were lower than 2019. Similar data were obtained for flowering. However, the time elapsed between the fruit's formation and its ripening and ending varies by year. The duration of activity changes with fruit, which is an important criterion for planting designs in landscape architecture studies; it is seen 33 days more in 2020 than in 2019. Considering the months of April-May-June when this process was experienced; it has been determined that monthly average, maximum and minimum temperatures are lower in 2020. On the contrary, the total amount of precipitation is higher in the same period. Climate data also affected the yellowing and shedding times of the leaves. Yellowing of leaves in 2020 is 22 days compared to 2019; the start of leaf fall also occurred 44 days earlier. In this process, the monthly average, minimum, and maximum temperature values realized in July-August-September-October were higher in 2020 compared to 2019. In the same period, the total amount of precipitation is 5 times lower in 2020 compared to the previous year.

CONCLUSION

Climate change due to global warming negatively affects plant material as well as many other materials in the world. This situation harms the economy of countries as well as a decrease in the visual quality of ornamental plants. Ornamental trees are used with their aesthetic features in landscape architecture applications; the visual contribution of the plant in design is gaining value.

Ficus carica and *Morus alba* 'Pendula' species, which are used extensively in the green areas of the campus and are members of the Moraceae family, in the study carried out between January 2019 and October 2020 at Iskenderun Technical University Central Campus, located in Iskenderun, the district of Hatay province on the Mediterranean coast. Monitored in terms of features; its relationship with climatic data has been revealed.

According to all data, climatic factors affected the phenological characteristics of both plants. In *Ficus carica*, the first leaf bud formation, leaf emergence, flowering, yellowing, and shedding of leaves were affected mostly by monthly average temperature, monthly maximum, and minimum temperatures. The total amount of precipitation in the relevant months decreases the fruit's activity period; it has been the factor accelerating the yellowing and shedding of leaves. In the *Morus alba* 'Pendula', the effect of climate factors is observed more in the fruit's activity process. In this process, monthly average temperature, monthly maximum, and minimum temperatures, and total rainfall are effective. The total amount of rainfall and temperature increase in the relevant months were factors that accelerated the yellowing and falling of the leaves. The data obtained also contributed to the determination of the visual activity period, which is important in the creation of planting designs in landscape architecture.

When the data obtained as a result of the study are evaluated, attention is drawn to the following issues.

* Taking all necessary measures to prevent global warming is of great importance for the sustainability of natural resources and quality of life.
* Green areas are one of the areas that contribute to the reduction of global warming. For this reason, the amount of green space should be searched especially in the urban area; planting should be given importance.
* Species selection should be given importance for trees that have an important role in this regard.
* It should be ensured that phenological observations are made for many years to reveal the aesthetically efficient period of the tree species to be used in urban green areas.

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CHAPTER 7

EFFECT OF DIFFERENT TREATMENT ON SEEDLING EMERGENCE OF *CENTAUREA PTOSIMOPAPPA*, A WILD ENDEMIC PLANT WITH ORNAMENTAL POTENTIAL

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INTRODUCTION

Biological diversity is also important, besides the historical and cultural wealth of the countries. The Asian populations were characterized by a higher level of genetic diversity than the European populations. Turkey, despite having an area of about one-fifteenth of the total area of European countries, plant diversity is as much as European countries. European countries other than Turkey possess 12,000 species, of which 2750 are endemic, while Turkey has an estimated 9000 species, of which 3000 are endemic (Ekim et al., 2000).

The province of Hatay is a region with high biodiversity, located at the eastern end of the Mediterranean Region, with coastal dunes, plains, scrub, and forest areas. Amanos Mountains are characterized by a high genetic diversity with many populations considered genetically unique. There are 107 families, 520 genus, and approximately 1580 taxon in this region. According to the Flora of Turkey, there are 175 endemic totally 1246 plant species in Hatay province. According to current studies, there are 1861 taxa in total in Hatay and 223 of these taxa are endemic (Kayıkçı & Oğur, 2012).

The genus *Centaurea* has about 800 species with the Mediterranean area and SW Asia as its centres of diversity (Wagenitz & Hellwig, 1996). The genus *Centaurea* is one of the larger genus in Turkey, represented by 187 taxa, of which 114 are endemic (endemism ratio: 60.9%) (Guner et al., 2000). The high endemism ratio shows that Turkey is one of the gene centres of the genus. *Centaurea* species are used as ornamental plants (Figure 1), forage plants, medicinal plants in the industry, food, and fire igniter. "Sweet Sultan", a variety of *Centaurea moschata,* is a commercial variety grown as an ornamental plant in the world and developed from the genetic resources of Turkey.

Figure 1: *Centaurea* cultivars used as ornamental plants; Black Ball (URL-1), Blue Boy (URL-2), Sweet Sultan (URL-3)

Centaurea ptosimopappa is an endemic species widespread and frequent locally in the Amanos and Casus Mountains. Its name is “Meşe Sarıbaşı” in Turkish. This shrub is having 100-180 cm in height, with ascending glabrous branches and yellow flowers. Achenes are 4-5 mm and pappus is very deciduous. It shows from 150 m than 1600 m altitude distributions around *Pinus* and *Quercus* forests (Davis, 1975). The highest value of total phenolic contents (mg gallic acid equivalent per gram) was detected in aqueous extract of *C. ptosimopappa* (Formisano et al., 2008: Erol-Dayi et al., 2011).

The requirements for seed germination are specific for each *Centaurea* species (Celik, 2006; Uysal et al., 2006; Celik & Yücel, 2008; Okay & Günöz 2009; Türkoglu et al., 2009) depend on environmental (Yankova-Tsvetkova et al., 2018; Eskin et al., 2019) and internal physiological factors (Okay & Günöz, 2009) which as dormancy, seed development etc. Each species is basically characterised by a temperature range over which germination is possible, sometimes responding differently to alternating temperature conditions (Ebadi et al., 2014), and may be linked to the climatic and ecological conditions to which the species is adapted and has grown (Turkoglu et al., 2009; Nosratti et al., 2017). Other factors may also affect the germination requirements of a species, e.g. pH, light, and moisture contents (Eskin et al., 2013), as well as the amount of time during which seeds are exposed to dry after-ripening (Eddleman & Romo, 1988).

There are very few seed studies of the species included in the *Centaurea* genus for cultivation. In most of these studies, the germination performances of the species are determined (Celik, 2006; Uysal et al., 2006; Celik & Yücel, 2008; Okay & Günöz, 2009; Turkoğlu et al., 2009; Eskin et al., 2013; Eskin et al., 2019). Cultivation of these species is absolutely necessary, whether for use as ornamental plants or for medicinal purposes. In addition, reproduction techniques should be studied for protection purposes in order to prevent the danger of extinction of endemic species. One of the best ways to protect endemic species is to determine their propagation method. The emergence studies of endangered species are the cornerstone of starting in-situ and ex-situ conservation studies that will enable us to sustain and develop natural populations on a large scale. With this study, it was aimed to determine the reproduction methods by the seed of the endemic *Centaurea ptosimopappa* species that spread in the flora of Hatay. For this reason, the effect of pre-sowing seed applications on seedling development was investigated in *C. ptosimopappa*.

MATERIAL AND METHOD

Source of collected seeds

Achenes of *C. ptosimopappa* were collected at maturity stage from randomly chosen plants that were growing in Amanos Mountain, Turkey, in July 2017. Achenes were extracted from the pappus by hand; care was taken to not damage the seed coat. The harvested seeds were dried at room temperature for 5 days in a well-ventilated place without sunshine, then selected, and stored in a refrigerator (4 ºC) until they were used for the experiment. The 1000 seed weight (7.2 g) was observed before seedling emergence tests (Figure 2A,2B).

Pre-sowing treatments

Pre-sowing treatments were performed using different concentrations of GA_3 and KNO_3. Two levels of GA_3 (250 and 500 ppm) and one level of KNO_3 (3%) were used for seeds pre-sowing. In order to determine the response of *C. ptosimopappa* to GA_3 and KNO_3, achenes were placed on filter paper that was moistened with 20 mL solution of GA_3 (250 and 500 ppm) or KNO_3 (3%). The achenes were kept in the relevant solution at 25 ± 2 ºC for 24 hours (Figure 2C).

Figure 2: Flower (A), seeds (B) and sample of presowing seed treatments (C) of endemic *C. ptosimopappa* species

Emergence test

After the treatments, emergence tests were conducted after surface drying and set up with 3 replications of 25 seeds in each treatment. To determine the effect of pre-sowing treatments on seedling emergence, seeds were sown at 0.2 cm depth in perlite stuffed plastic boxes (19×9×6 cm length × width × height). Seedlings were grown in laboratory conditions at 23 ± 2ºC for 25 days. In the control application, untreated seeds were planted in perlite medium at two different depths (0.2 and 1.0 cm) and in peat moss medium (1.0 cm). Here, it is aimed to determine the reaction of the species to light by using the light reflecting feature of perlite. Emerged seed counts (hypocotyl arch visible) were made daily until the percentage of emerging seedlings had stabilized in all treatments and final emergence percentage and mean emergence time were calculated.

Traits measurement

When the percentage of emergence had stabilized in all treatments, seedlings were sampled for traits. Then, emergence percentage, mean emergence time (Orchard, 1977), emergence index (Maguire, 1962) coefficient of variation of emergence time (Kader, 2005), coefficient of velocity of emergence (Kader, 2005), root length (cm), leaf length (cm), and time to first true leaf formation (day) (Virk et al. 2020) were calculated at the end of the trial. Five seedlings with three replicates were randomly selected from seedling samples. The root length, and leaf length as well as the total length were measured using a digital calliper.

Statistical analysis

Mean data for each character was evaluated by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test when the P value less than 0.05 was considered significant. Percentages were arcsine transformed prior to analysis and were present the untransformed data in the figures.

RESULTS AND DISCUSSION

The percentage of emergence was determined as a significant ($P \leq 0.05$) difference between pre-sowing treatments and control seeds (Figure 3). GA_3 (250 and 500 ppm) and KNO_3 treated seeds reached at 95%, 99% and 97% respectively, while control peat seed had 19% seedling emergence (Figure 3). GA_3 (500 ppm) pre-sowing treatment was superior throughout the duration of the experiment. There was little difference between both pre-sowing treatments, but a significant difference between the treated and control seeds were observed (Figures 3 and Figure 4).

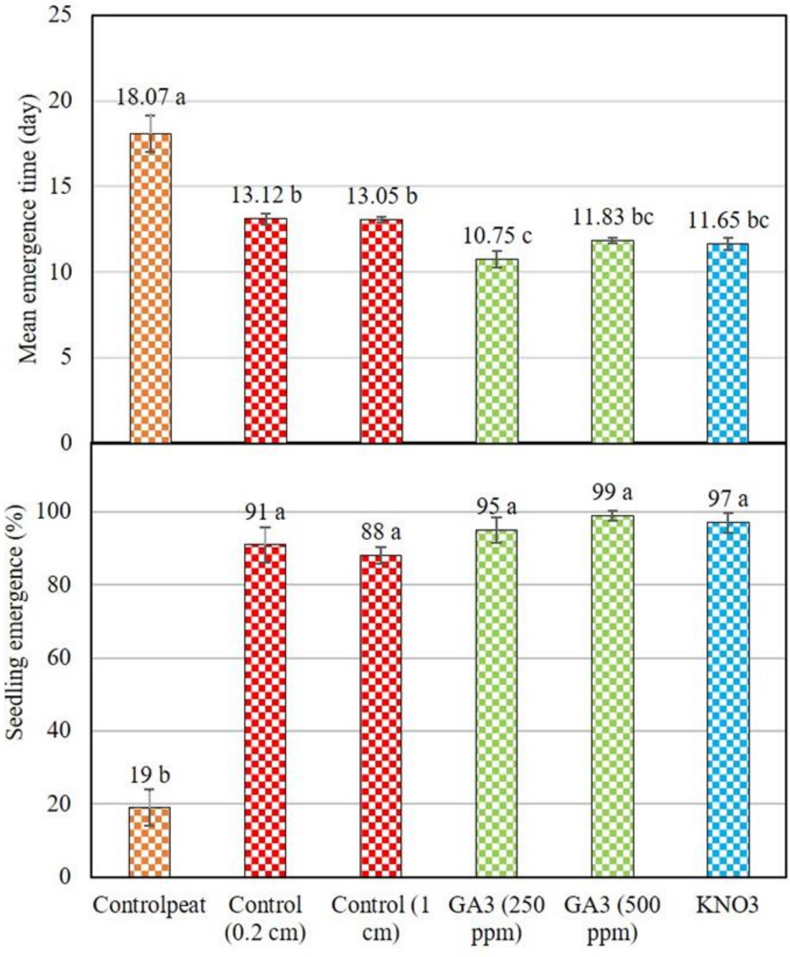

Figure 3: Emergence percentage and mean emergence time of *Centaurea* seeds
Error bars represent ± standard error; n = 3

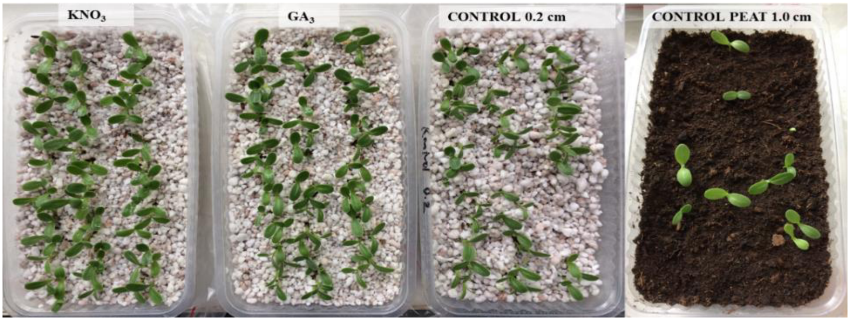

Figure 4: Emerging performance of pre-sowing treatments in *Centaurea* seeds 18th day after sowing

Mean emergence time was recorded in GA_3 (250 ppm) treatment as 10.75 days. While it is in the same group with GA_3 (500 ppm) and KNO_3 treatments, it is significantly higher than all control seeds (Figure 3).

Emergence index, and coefficient velocity of emergence increased and coefficient variation of emergence time decreased with effect of pre-sowing treatments (Figure 5) and all of them were statistically different compared to control peat. While the highest coefficient velocity of emergence (9.34) was recorded in seeds treated with GA_3 (250 ppm), the highest emergence index was recorded in seeds treated with GA_3 (250 and 500 ppm) and KNO_3. Control perlite seeds worked just as well as the treated seeds. However, control peat seeds had the lowest values. The coefficient variation of emergence time, on the other hand, has lower values in treated seeds with GA_3 (250 ppm) compared to the control peat (Figure 5).

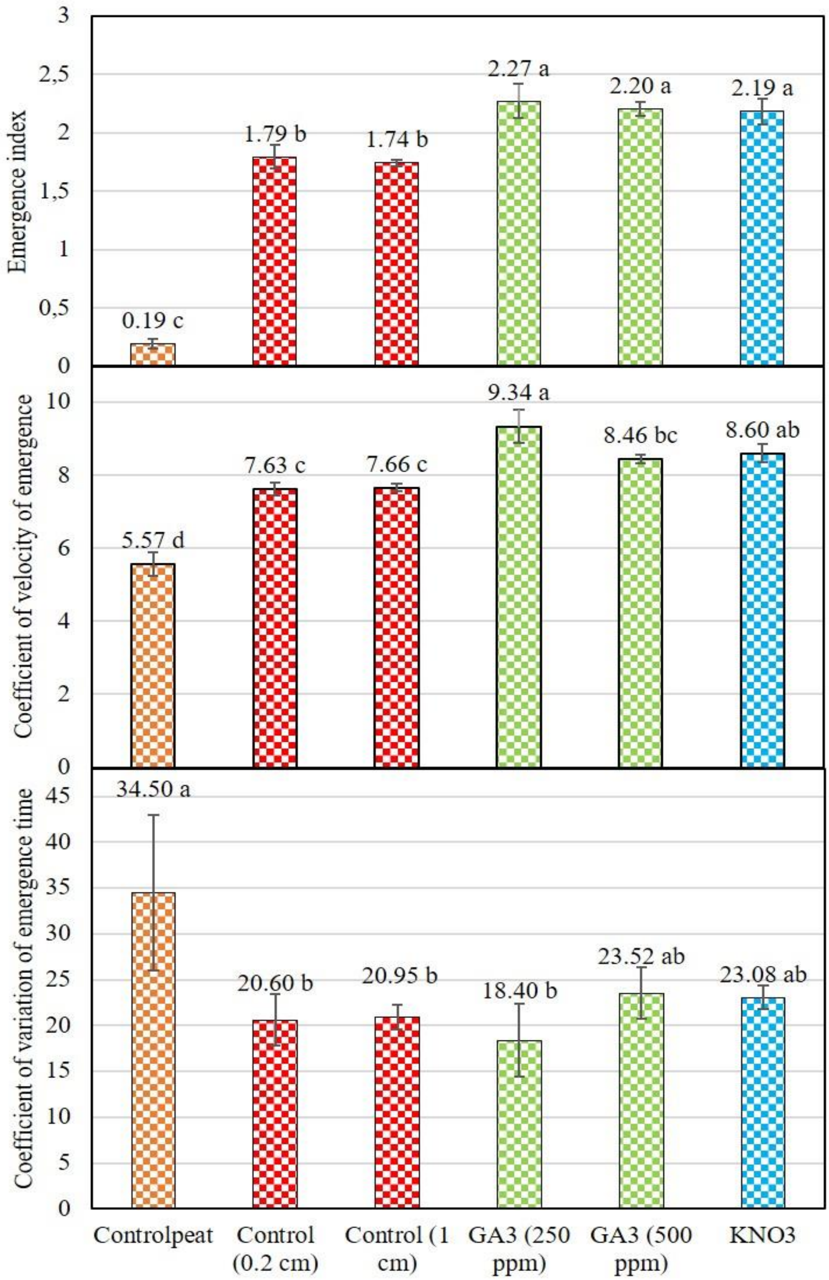

Figure 5: Effect of treatments on emergence index, coefficient velocity of emergence and coefficient variation of emergence time

Seedling root length, leaf length and time to first true leaf formation are presented in Figure 6.

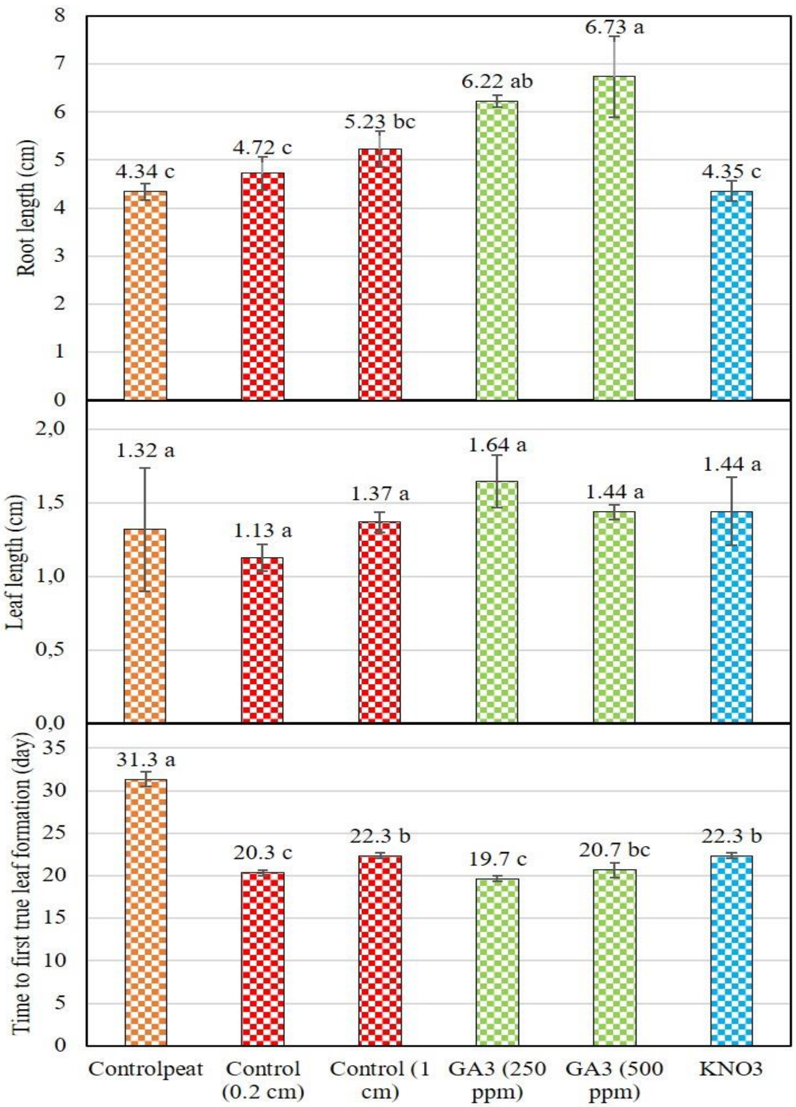

Figure 6: Effect of treatments on root length (cm), leaf length (cm), and time to first true leaf formation (day)

Maximum seedling root length (6.73 cm) was obtained under pre-sowing treatment with GA_3 (500 ppm), minimum root length (4.34 cm) was recorded under control peat seeds. Although, GA_3 (250 ppm) has the highest value in leaf length, there was no statistically significant difference between the other applications (Figure 6).

In general, light was not required for seed germination in many *Centaurea* species (Nosratti et al., 2017; Atasagun & Aksoy, 2018), but, some species require light for germination (Riemens et al., 2004; Celik, 2006; Celik & Yücel, 2008; Ebadi et al., 2014; Eskin et al., 2019). Another factor affecting seed germination is temperature. Researchers have reported that seed germination of other species of *Centaurea* genus was highest at constant temperatures of 15 ºC to 25 ºC (Turkoglu et al., 2009; Ebadi et al., 2014; Nosratti et al., 2017). There is no information available on germination temperatures of *Centaurea ptosimopappa*. Therefore, it has been tested at 25 ºC degrees, which is suitable for other species.

Studies on *Centaurea* species have generally been carried out to determine the effect of different applications on seed germination (Celik, 2006; Uysal et al., 2006; Celik & Yücel, 2008; Okay & Günöz, 2009; Turkoglu et al., 2009; Eskin et al., 2013; Eskin et al., 2019). However, in order for these species to be included in culture, it is necessary to work on the seedling emergence and seedling quality.

In the present study, seed priming through the use of GA_3 (250 and 500 ppm) had a positive effect on emergence percentage, mean emergence time, emergence index, coefficient velocity of emergence and root length and these results are in agreement with the findings of some previous studies, revealing the effects of gibberellic acid on the seed germination of different *Centaurea* species (Göztaş, 2008; Atasagun, 2012). Seedling emergence percentages were found to be very low (15% in control, and 43% in 100 ppm GA_3) in some species (*C. tchihatcheffi*). It can be said that this is due to low GA_3 treatment doses (10 and 100 ppm) and the genetic background of the species (Okay & Günöz, 2009).

The application of KNO_3 had a similar and stimulatory effect on the seedling performance of *C. ptosimopappa*. It has been shown that KNO_3 increase germination and emergence performance in some *Asteraceae* species (Samfield et al., 1990; Kanatas et al., 2020) and decreased in others (Celik, 2006; Uysal et al., 2006; Celik & Yücel, 2008). Potassium nitrate enhances breaking dormancy in various species seeds which reduces plant growth regulators such as abscisic acid (Yang et al., 2007). KNO_3 application provided that seeds of *C. ptosimopappa* species had a high emergence percentage and other parameters (except for root length and leaf length) as well as GA_3 applications in the present work. It is thought that this situation may be caused by some genetic and environmental factors.

While the treatments caused improvement in emergence percentage, it also caused a decrease in mean emergence time. Mean emergence time 18.07 days in control peat seeds which were significantly reduced to 10.75 days in seeds treated with GA_3 (250 ppm). Similarly, Göztaş

(2008) reported that the GA_3 treatment (1000 ppm) was superior in reduction of mean emergence time. Okay & Günöz (2009) found that GA_3 treatment reduced mean emergence time of *C. tchihatcheffi* while hydro priming has a negative influence on mean emergence time.

The sowing depth influenced *C. ptosimopappa* ability to emerge. The control (0.2 cm) depth recorded lower emergence percentage than control 1.0 cm sowing depth. Similar results have been reported in other plants for example Watson & Renney (1974) who found that seedling emergence was decreased with increased sowing depth in *Centaurea diffusa* and *Centaurea maculosa.* Also Nosratti et al. (2017) reported that seedling emergence decreased with increase in sowing depth in *Centaurea balsamita.* Again, Nosratti et al. (2017) reported that sallow sowing (0 cm) causes higher emergence percentage than sowing at 1.0 cm depth. This situation is thought to be related to the seeds receiving light. From this point of view, it can be said that *Centaurea ptosimopappa* seeds should not be planted deeply, even in perlite medium. Deep sowing can significantly reduce crop emergence and other parameters, especially in peat moss and soil medium. The deeper the seed is sown the more strength it needs to push its shoots above the soil surface.

Some seedling quality characteristics were improved with seed applications with GA_3 and KNO_3 in *Centaurea ptosimopappa* species. Especially, more successful results were obtained from 250 ppm GA_3 application in terms of root length, leaf length and time to first true leaf formation compared to control applications studies on cultivation and seedling quality in *Centaurea* species are quite insufficient. It has also been reported by Virk et al. (2020) in cotton.

In conclusion, among the factors examined for the germination process of *Centaurea*, species temperature and osmotic potential seem to be the critical factors. Seedling emergence of *C. ptosimopappa* tested regimes in the different medium and pre-sowing treatment might be helpful for its successful seedling establishments practice for culturalization. It was determined that *C. ptosimopappa* seeds emerged more successfully in perlite medium than peat medium. In order to obtain more seedlings, it may be recommended to take the seedlings into perlite + soil + peat mixture after seedling emergence in the perlite medium. In addition, sowing depth affects the seedling emergence percentage and other parameters. Hence, this information would be beneficial for light request of *C. ptosimopappa*.

We can summarize the obtained results as follows:

1) It was concluded that seedling emergence characteristics of *Centaurea ptosimmopappa* were higher than the other *Centaurea* species.

2) Both gibberellic acids (250 and 500 ppm) were more effective on seedling emergence characteristics compared with control and control in peat moss,

3) Also, treatment of KNO_3 was effective on seedling emergence and seedling characteristics. This information will contribute to the use and culture of the species as an ornamental plant.

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CHAPTER 8

EFFECT OF PLANTING DATE AND DISTANCE ON TURFGRASS ESTABLISHMENT WITH *ZOYSIA JAPONICA* 'MEYER' BY VEGETATIVELY IN MEDITERRANEAN CONDITIONS

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INTRODUCTION

In the world and Turkey is increasing the need for green space in urban areas with increased structuring by time. The use of turfgrass species that are suitable for climatic conditions and can be established quickly in green areas whose value is increasing day by day is gaining importance. Grass plants, which can create large, homogeneous green areas and at the same time contribute to the distinctive appearance of ornamental plants, used in landscape areas, provide aesthetic and beautiful views to the area. Also grass plants are widely used in structural environments, parks, gardens, golf courses, football fields etc. sports fields, airports, cemeteries, highway slopes etc. environments, and they give these areas functionality and aesthetic appearance. In this direction it is increasing the importance of the work done on the lawn in Turkey. Bermuda grass is the most widely used specie of turfgrass with a very good adaptation in establishing turfgrass area under the conditions Mediterranean climate. However, Bermuda grass needs at least 6-8 hours of full sunshine in order to develop and grow well and its cold tolerance is very poor, so it cannot perform well in areas with shade and semi-shade conditions, especially in transition climates (Sever Mutlu et al., 2011). It is observed that mixtures of some types of cool season grasses with relatively better shade tolerance in such areas are widely used in the region. (Sever Mutlu et al., 2011). However, this situation negatively affects the speed of establishment in the area by stressing types of cool season grasses species due to the high temperatures in the summer. Therefore, the green areas which need to be re-established increase the maintenance cost significantly.

Therefore, it is important to test in Turkey of alternative turfgrass species to Bermuda grass that can assume different functions with different growth characteristics and appearance in hot climatic conditions is important. In recent years, *Zoysia japonica* has come to the fore as one of the alternative grass types, especially for the Mediterranean region conditions.

Zoysia japonica (Japanese grass) is an important type of grass that is used in home gardens, parks, golf and sports fields in many countries of the world with its highquality grass texture (Beard, 1973; Richardson, 2003; Sever Mutlu & Temizel, 2013). Japanese grass, which has the best tolerance to low temperatures in winter among warm season grass types, can survive even in sub-zero temperatures (Emmons, 2000; Dunn, 1991; Patton et al., 2007; Sever Mutlu & Temizel, 2013). In this regard, Japanese grass has a significant potential not only for Aegean and Mediterranean coastal regions in Turkey but also for green areas that will be established in the transition zone climate. Japanese grass is a type of grass that is cheaper and easier to maintain due to its excellent tolerance to high temperatures, drought, weeds, diseases and pests and stepping compared to cool season grass types (Youngner, 1961; Brian et al., 1981; White et al., 2001; Sever Mutlu & Temizel, 2013). Due to its good shade tolerance (Morton et al., 1991; Emmons, 2000), Japanese grass is an excellent choice for parks with semi-shade conditions that cannot get full sun, home gardens, golf and football fields as an alternative to Bermuda grass as a warm season grass type. Despite considerable potential for

Mediterranean and transitional climate regions, Japanese grass is a type of grass recognition began in recent years in Turkey. When the literature was examined, the current studies on this species were studies conducted especially in different states of the USA (Beard, 1973; Hall et al., 1998; Richardson et al., 2003; Patton et al., 2004; Zuk & Fry, 2005; Okeyo et al., 2011). It was seen that the number of studies on the performance of this species and the grass area facility in the Mediterranean climate conditions (both our country and other Mediterranean countries) was limited (Volterrani et al., 1997; Croce et al., 2001; Geren et al., 2009; Sever Mutlu et al., 2011; Pompeiano & Volterrani, 2012). This situation was caused *Z. japonica* grass species recognition and use less limited in Turkey. Therefore, it was important to work on the optimum conditions required to establishment of turfgrass area in Mediterranean climate conditions with this grass type. The use of Japanese grass in different areas such as golf fields, football fields, home gardens and parks will increase significantly by providing the optimum conditions for a fast establishment. Especially good adjustment of seeding and planting time and the methods used affect the speed of establishment on this type (Richardson et al., 2003; Patton et al., 2004; Temizel, 2014).

Despite the introduction of new varieties of *Zoysia japonica* (including seeded varieties) to the lawn industry, 'Meyer', one of the oldest varieties, was still one of the most widely used vegetative varieties in transition climatic regions, especially in the USA, due to its superior characteristics such as low maintenance and tolerance to low temperatures below zero (Christians & Engelke, 1994; Temizel, 2014). Since it does not produce seeds, the 'Meyer' variety must be vegetatively established in the field by planting method (plugging) with grass seedlings grown in viols, sprigging with stolon and rhizome pieces or one of the roll grass methods (Temizel, 2014). Although the roll grass method seems more attractive at first glance, because it is expensive other vegetative methods are preferred more and studies focus on them (Carroll et al., 1996; Richardson & Boyd, 2001; Richardson et al., 2003; Sladek et al., 2011; Okeyo et al., 2011; Temizel, 2014). Although the preferred planting time varies according to the method applied, it can take two years to obtain a dense grass cover in planting with grass seedlings or stoloning (sprinkling planting) methods (Hume & Freyre, 1950; Sifers et al., 1992; Emmons, 2000; Temizel, 2014). In this sense, determining the optimum planting time and method is very important in order for the turf to be established successfully in the field before the winter season and to prevent the grass cover from being affected by the winter cold, especially in the transition areas. Thus, with a successful establishment of turfgrass area, maintenance costs can be reduced and it may be possible to recommend Japanese turf as an alternative to bermuda grass in the region, especially in semi-shade and shade conditions. The aim of this study is to find the optimum planting time and planting density for the establishment of turf by planting with grass seedlings grown in viols and vegetatively using "Meyer" variety of *Zoysia japonica* species in Mediterranean conditions.

MATERIAL AND METHOD

The study was carried out in the experimental area prepared in the Akdeniz University campus research application area, between June and December 2012 (Figure 1). As vegetative material, *Zoysia japonica* 'Meyer' variety, approximately eight weeks before planting, was clonally propagated from its stolons under greenhouse conditions and rooted seedlings (plug) with an average diameter of five (5) cm were used (Figure 2).

Figure 1: General photo of the trial area (Original 2012)

Figure 2: Seedling of *Zoysia japonica* 'Meyer' variety

The study was carried out with 2 factors and 3 replications in split plots experiment design in random blocks, and the effects of planting time (main plot) and planting distance (divided plot) on establishment of grass area in the "Meyer" variety were evaluated. The trial pattern plan for this study is given in Figure 3.

The trial area is under the influence of the typical Mediterranean climate, with hot and dry summers and mild and rainy winters. The highest and lowest temperature, average humidity, monthly total precipitation values for the period of June 2012 - July 2013 in the Antalya Region are given in Table 1.

Table 1: Meteorological data of antalya province, where the study was conducted, for the period of June 2012 - July 2013

Month	Maximum temperature (°C)	Minimum temperature (°C)	Average humidity (%)	Total Precipitation (mm)
June	43	14,7	61,2	31,4
July	42,9	21,2	54,3	0
August	40,5	21,6	44,4	0
September	39,2	17	58,8	0
October	35,6	13,9	68,5	128,4
November	27,5	5,9	70,1	31,6
December	21,7	2,5	75,9	518
January	14,9	7,2	72,4	461
February	17,1	7,7	72,5	80,2
March	18,2	8,7	66,9	26,8
April	23,5	12,5	65,9	66,4
May	28,2	17,5	64,6	60,4
June	29,5	22,2	63,1	0
July	33,4	25,3	55,1	0

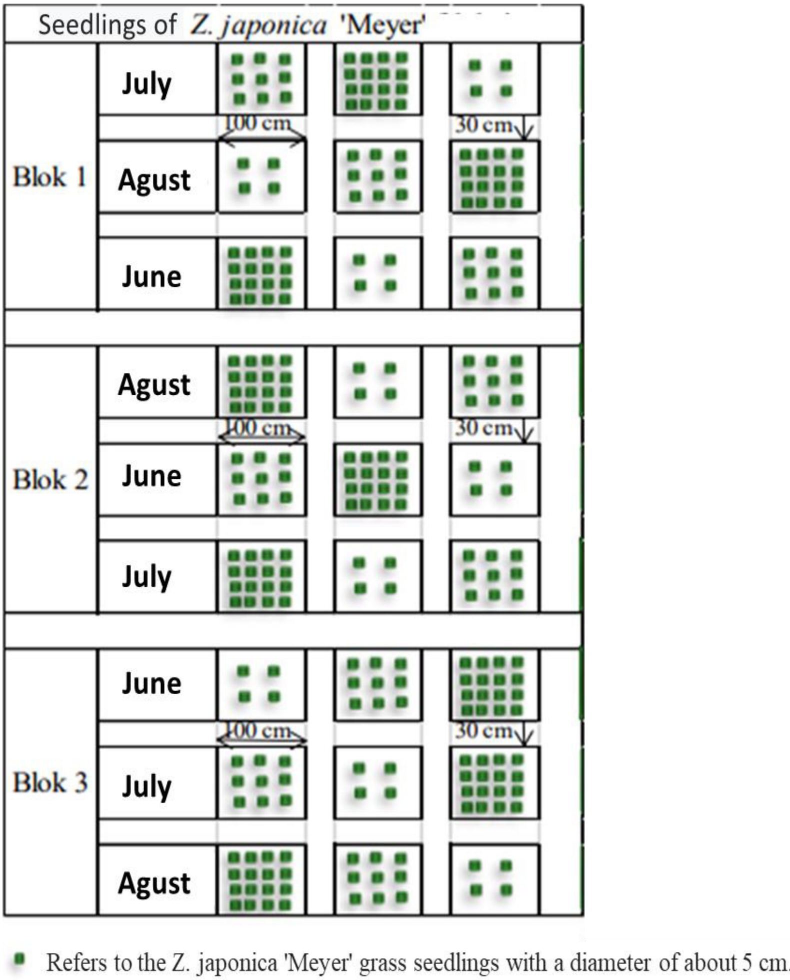

Figure 3: Trial pattern plan

The results of the soil analysis of the soil sample taken from the trial area, made in the Western Mediterranean Agricultural Research Institute Regional Soil, Plant Water and Fertilizer Analysis Laboratory, are given in Table 2. Soil analysis was made according to the analysis method and standards of the Ministry of Agriculture and Forestry.

Looking at the chart, it is seen that the trial area has a clay loam, high alkali and high lime soil structure.

Table 2: Soil characteristics of the trial area

Soil Analysis Results		
pH (1:2,5)	8,7	Strong Alkaline
Lime (%)	29,7	Too Much Chalky
EC micromhos/cm (25 °C)	228	Saltless
Sand (%)	37	
Clay (%)	34	Clay Loam
Mile (%)	29	
Org. Matter (%)	1,6	
P ppm (Olsen)	31	
K ppm	407	
Ca ppm	5150	
Mg ppm	337	

Z. japonica 'Meyer' seedlings were planted at 3 different planting distances on 20 June 2012, 20 July 2012 and 20 August 2012 in the trial plots prepared in 1 m x 1 m dimensions in the field. 25 cm (16 pieces / m²), 34 cm (9 pieces / m²) and 50 cm (4 pieces / m²) were used as the planting distance between seedlings (Figure 4a, Figure 4b, Figure 5).

Just before planting, 15N-15P-15K fertilizer was applied in a net amount of 5 g N per m². Two weeks after planting, 2.5 g / m² N was given to the parcels regularly at intervals of two weeks by using ammonium sulphate fertilizer. Two weeks after planting, 2.5 g / m² N was given to the parcels regularly at intervals of two weeks by using ammonium sulphate fertilizer. The last fertilizer application was made on September 15th. The parcels have been irrigated 3 times a day since planting. In the following weeks, the frequency of irrigation was reduced and regular irrigation was carried out in a way to prevent the plants from getting stressed. Grass is mown weekly from 4 cm height during the active growth period. In order to control the weeds between the plots, glyphosate (0.28 kg ha^{-1}) was applied every 15 days with a pulverizer. The weeds formed in the plots were cleaned manually and the pressure of the weeds on *Zoysia japonica* was prevented.

(a) (b)

Figure 4: a: According to the planting distance of 50 cm, 4 *Zoysia japonica* 'Meyer' seedlings were planted in 1 m² area, **b:** 9 *Zoysia japonica* 'Meyer' seedlings were planted in 1 m² area according to the planting distance of 34 cm

Figure 5: 16 *Zoysia japonica* 'Meyer' seedlings were planted ın 1 square meter area according to 25 cm planting distance

After planting, the following data were taken from each parcel:

Establishment rate: The rate of establishment of *Zoysia japonica* grass species was determined every two weeks during the study from seed planting. Establishment rate is the evaluation of the area covered with grass plants visually (%). This value, which is determined using a scale of 0–100, gives the grass plant the speed to develop a mature grass texture covering the whole parcel from planting.

Turfgrass quality: Evaluation of turfgrass quality is a combination of reaction to stresses that caused by the color, homogeneity (uniformity) density, texture (texture) and environmental and / or disease etc of the grass texture (Turgeon, 1985). Visually assessed using a 1-9 quality rating scale. In this scale, 1.0 represents complete yellowing (dormancy or death), 6.0 represents the minimum acceptable turf quality, and 9.0 represents ideal shoot density, texture, color and homogeneity, perfect or ideal quality. Data were taken every 15 days.

Turfgrass density: It was determined by counting the existing grass shoots in the grass molds removed with an 8 cm diameter grass profile sampling device (cup cutter) from 3 points randomly selected from each parcel (Figure 6). After the measurements were made, the grass profiles were placed back to their original places. The counts were made in November 2012, just before grasses entered the dormant period. Thus, the rate of change in grass density according to planting time and planting frequency was determined.

Figure 6: Shoot count process

The data obtained were analyzed by using the variance analysis method with PROC GLM (SAS Institute, 1999) program. LSD (0.05) values were calculated by comparing the means with the least significant difference (LSD) test preserved according to Fisher method.

RESULTS AND DISCUSSION

As a result of the statistical analysis, the differences between planting times and planting distances were found important in terms of establishment speed (Table 3, Figure 7). The differences between planting distances at all tested planting times were found statistically significant and the rate of establishment increased significantly as the planting frequency increased. Considering all planting times and planting distances together, 'Meyer' seedlings, which were planted with a planting distance of 25 cm (16 pieces / m²) in June, were statistically the fastest establishment in the field, and after only two months after planting, approximately 96% turfgrass cover formed. The parcels planted with a planting distance of 34 (9 pieces / m²) and 50 cm (4 pieces / m²) in June, on the other hand, reached to 90% and above coverage in the 10th and 12th week, respectively.

The parcels planted with a planting distance of 25 (16 pieces / m²), 34 (9 pieces / m²) and 50 cm (4 pieces / m²) in July reached to 90% and over coverage respectively in the 12th, 14th and 17th week. (Table 3, Figure 7). In June and July plantings, although the success of establishing 90-100% in the same growing season in all planting distances, the same success could not be achieved at any planting distance planted in August. The parcels planted with 25, 34 and 50 cm planting distances in August reached to 71%, 52% and 31% coverage, respectively, at the end of the same growing season (20 December 2012). The parcels planted with 25, 34 and 50 cm planting distances in August reached to 71%, 52% and 31% coverage, respectively, at the end of the same growing season (20 December 2012) (Table 3, Figure 7). The parcels planted in August could only reached to 90% and above turfgrass coverage 39-41 weeks after planting in the second growing season.

Carroll et al. (1996) reported that 5 cm diameter grass seedlings can be used at 15 cm intervals in their study. Gibeault et al. (1988) suggested that in their study with the 'El Toro' variety, 5 cm grass seedlings should be planted using a planting distance of about 23 cm (9 inches) from their centers as a planting method. Unruh et al. (2011) reported that Japanese grass seedlings are usually planted 20-30 cm distance from their center. Similarly, within the scope of this thesis study, the fastest turfgrass establishment rate of *Zoysia japonica* species in Mediterranean conditions was obtained in June and with a planting distance of 25 cm. Sladek et al. (2011) also reported that in *Zoysia matrella* species, grass seedlings planted in the field at 15.2-30.5 cm intervals in the late spring and early summer period provided the fastest turfgrass establishment.

Beard (1973) suggested a planting distance of 15-40 cm as the most suitable planting distance for Japanese grass seedlings. Within the scope of this thesis, the results obtained; It shows that 25-50 cm planting distances can be used depending on the planting time. For example, in the plantings in June, 100% grass cover was reached at all planting distances of 25, 34 and 50 cm long before the growing season was over. On the other hand, in July plantings, at the end of the growing season, when the grasses entered dormancy, nearly 100% grass cover was provided with only 25 cm planting distance. Henry et al. (1988) reported that the stolon and grass seedlings planted at the beginning of the summer were completely established in the field after 3-4 months in their study using the 'El Toro' variety. It is seen in Table 3 and Figure 8 that all of the planting distances we made in June reached a 100% establishment speed in all planting distances after 12-16 weeks (3-4 months). Therefore, the data we found for June corresponds to the data of Henry et al. Miyachi et al. (1993) reported that the acceptable plant of zoysia grass with stolon and rhizome under the climatic conditions of Japan was obtained within 85 and 110 days after shoot growth started. Fagerness et al. (2002) reported that *Z. japonica* 'Meyer' variety was established at a rate of 90% after 116 days using the scatter planting method with stolon and rhizome fragments. In our study, "Meyer" variety was established in a shorter time with the seedling planting method. 'Meyer' variety covered 90% of the area after 60-75 days (8-10 weeks) and 90-119 days (12-17 weeks), respectively, according to the planting distance used in June and July (Table 3).

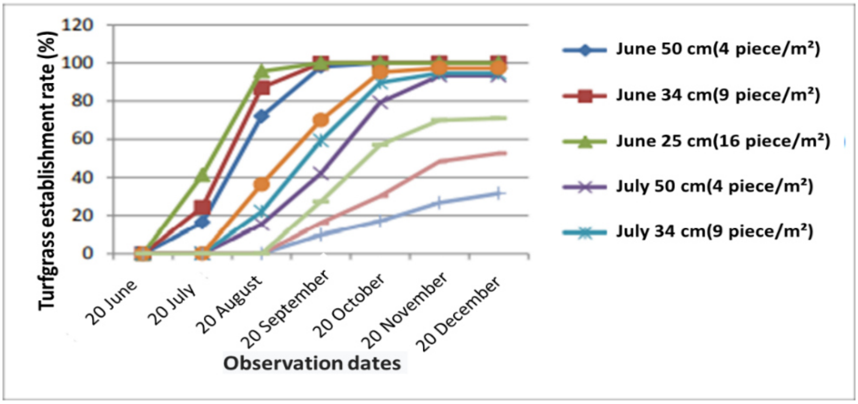

Figure 7: The average rate of the area covered with grass in the same growing season of *Zoysia japonica* 'Meyer' variety planted in June, July and August in Antalya in 2012 (%)

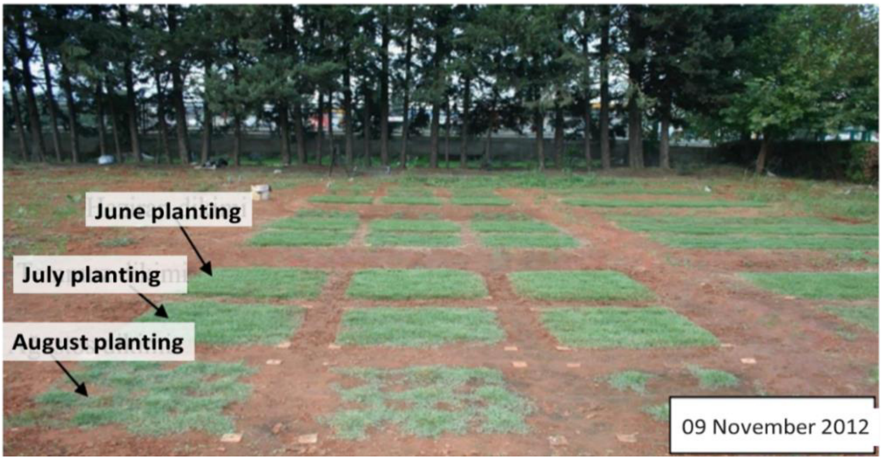

Figure 8: General view of seedlings of *Z. japonica* 'Meyer' variety planted in Antalya in June, July and August in 2012, before the dormancy period, in November 2012

Table 3: Average turf establishment rate of *Zoysia japonica* 'Meyer' variety planted in June, July and August in Antalya in 2012 on a weeks (%)

Planting time/Planting Distance		Turfgrass Establishment Rate (%)[x]														
		Time after planting (week)														
		4	6	8	10	12	14	17	33	35	37	39	41	43	46	48
June	50 cm – 4 piece/m²	16 e[y]C[z]	42 dC	72 cC	83 cdC	98 aB	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA
	34 cm – 9 piece/m²	24 cdB	62 bB	87 bB	92 bB	99 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA
	25 cm- 16 adet/m²	41 aA	80 aA	95 aA	99 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA	100 aA
July	50 cm – 4 piece/m²	15 eC	22 fC	42 eC	64 eB	79 cB	86 cB	93 bA	93 aA	93 aA	96 aA	99 abA	100 aA	100 aA	100 aA	100 aA
	34 cm – 9 piece/m²	22 dB	36 eB	59 dB	81 dA	89 bA	91 cAB	94 bA	94 aA	94 aA	97 aA	99 abA	100 aA	100 aA	100 aA	100 aA
	25 cm- 16 adet/m²	36 bA	54 cA	70 cA	87 bcA	95 abA	95 abA	97 abA	97 aA	97 aA	98 aA	100 aA	100 aA	100 aA	100 aA	100 aA
August	50 cm – 4 piece/m²	9 fC	13 gC	17 gC	23 gC	26 fC	26 fC	31 eC	50 dC	55 dB	70 dB	78 dB	88 cA	92 bA	96 aA	100 aA
	34 cm – 9 piece/m²	16 eB	24 fB	30 fB	39 fB	48 eB	48 eB	52 dB	61 cB	68 cA	80 cAB	88 cAB	92 bcA	95 abA	98 aA	100 aA
	25 cm- 16 adet/m²	27 cA	41 dA	57 dA	64 eA	70 dA	70 dA	71 cA	73 bA	80 bA	87 bA	94 bA	96 abA	97 abA	100 aA	100 aA

[x]Turfgrass establishment speed and rate were evaluated using a 0-100 visual scale, 100% in this scale indicates that the entire parcel is covered with grass.

[y]The planting distances shown in different small letters on each observation date differ from each other according to the Fisher method, according to the least significant difference (LSD) values preserved at the $P \leq 0.05$ level.

[z]The planting distances shown in different capital letters on each observation date, within each planting date, are different from each other according to the Fisher method according to the least important difference (LSD) preserved at the level of $P \leq 0.05$.

As a result of statistical analysis, the differences between planting times and planting distances in terms of grass density values were found to be significant (Table 4, Figure 9). Planting times / distances shown in different letters in Table 3 and Figure 9 are different from each other according to the least significant difference (LSD) values preserved at $P \leq 0.05$ according to the Fisher method. According to the turfgrass density counts made before the grasses entered dormancy in November, the highest number of shoots per unit area was provided by June plantings with approximately 206 pieces / 50 cm^2. This was followed by July and August planting dates, respectively. There was no statistically significant difference in density values between planting distances investigated in June (Table 4, Figure 9). The parcels planted with 25 and 34 cm planting distances in July are not statistically different from each other with 184 and 170 pieces / 50 cm^2 shoot density, respectively. Both of them formed statistically more dense shoots than 50 cm planting distance (153 pieces / 50 cm^2) (Table 4, Figure 9). In August, the differences between planting distances were found to be significant and the highest density value was achieved with a planting distance of 103 pieces / 50 cm^2 and 25 cm. This was followed by plots of 78/50 cm^2 and 49/50 cm^2, respectively, with a planting distance of 34 cm and 50 cm (Table 4, Figure 9). These results were in parallel with the turfgrass establishment speed and general turfgrass quality results. Especially since the parcels planted in June were established in a shorter time, it was able to provide the time to mature the shoots before dormancy and allow them to tiller. Plantings partially made in July and especially in August did not obtain enough time to allow the development of shoots before dormancy.

Table 4: Density data average of *Zoysia japonica* 'Meyer' variety planted at a distance of 25, 34 and 50 cm in June, July and August in Antalya in 2012, on 15 November 2012

Planting time	Plugging distance (piece/m²)	Density data (piece/50 cm²)
20 June	50 cm(4)	206,77 **a**
	34 cm(9)	205,55 **a**
	25 cm(16)	206,33 **a**
20 July	50 cm(4)	152,77 **c**
	34 cm(9)	170,55 **b**
	25 cm(16)	183,77 **b**
20 August	50 cm(4)	49,33 **f**
	34 cm(9)	78,77 **e**
	25 cm(16)	103,33 **d**

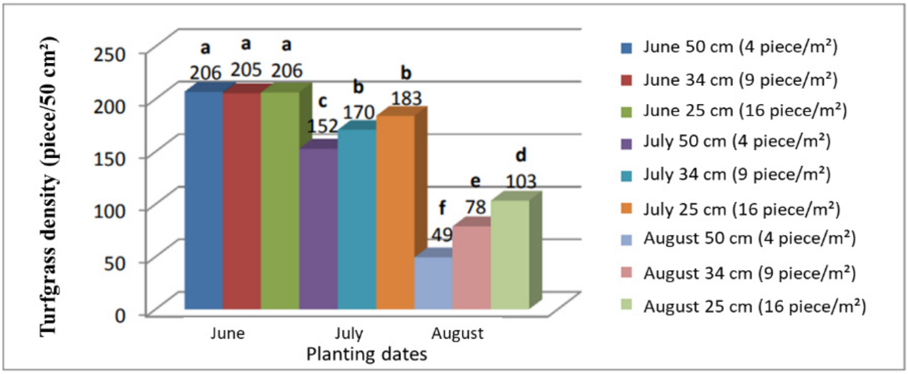

Figure 9: Turfgrass density values of *Zoysia japonica* 'Meyer' variety planted on 20 June, 20 July and 20 August 2012 at 25 cm, 34 cm and 50 cm planting distances on 15 November 2012 per unit area (50 cm^2)

Within the scope of this study in which the optimum seedling distance and seedling planting time were determined in the vegetatively plant of *Z. japonica* 'Meyer' variety, the grass qualities were examined every 2 weeks after planting, but the analysis results are summarized only for the 8th, 12th and 16th weeks and presented in Table 5. Planting times shown with different letters on each observation date in Table 5 and Figure 10 are different from each other according to the least important difference (LSD) values preserved at $P \leq 0.05$ according to Fisher method. Considering the average general turfgrass quality of all parcels since planting, there was no statistically significant difference between planting times and distances in the 4th week. However, as a result of the statistical analysis performed on the 8th and 12th and 16th weeks, the differences between planting times and planting distances in terms of turfgrass quality values were found to be significant (Table 5, Figure 10). Only 8 weeks after planting, the highest turfgrass quality was achieved with a 7.8 quality scale value in June-25 cm planting distance. The second best turfgrass quality was determined in the parcels planted at 34 cm planting distance in June with a quality scale of 6.7. On the same date, acceptable grass quality (6.0 quality scale value) could not be reached at other planting times and distances (Table 5, Figure 10). 3 months (12 weeks) and 4 months (16 weeks) after planting, except for the parcels planted in August, all other planting distances were acceptable and above ($\geq$ 6.0) turfgrass quality scale values were provided. 12 weeks after planting, the highest turfgrass quality with 8.2 quality scale value was determined in parcels planted in June and July with 25 cm planting distance. In addition, the quality difference between the 25 cm and 34 cm planting distances of these two months was not statistically significant, while both planting spacings provided better quality than the 50 cm planting distance. The parcels in which 25, 34 and 50 cm planting distances were used in August provided 3.5, 3 and 2.5 turfgrass quality, respectively, in the 16th week. Therefore, the plantings in August could not provide acceptable turfgrass quality before the dormancy period (Table 5; Figure 10). These results were parallel to turfgrass establishment speed and shoot density values.

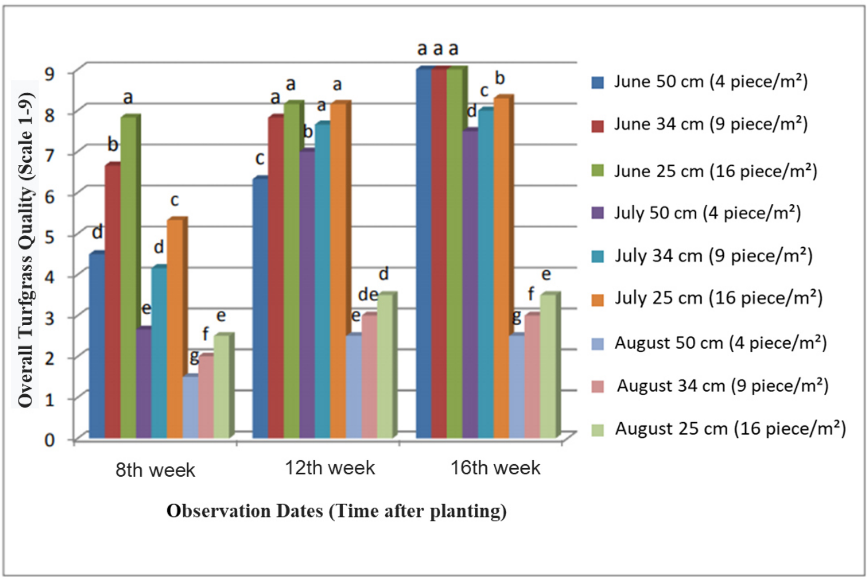

Figure 10: Quality changes of *Zoysia japonica* 'Meyer' variety during the trial period

Table 5: Average quality data of *Zoysia japonica* 'Meyer' variety planted in June, July and August in Antalya in 2012, on the basis of weeks from planting

Planting Time	Plugging distance	Quality Data (1-9) Time after planting (week)		
		8th Week	12th Week	16th Week
20 June	50 cm(4 piece/m²)	4,5 d	6,33 c	9 a
	34 cm(9 piece/m²)	6,66 b	7,83 a	9 a
	25 cm(16 piece/m²)	7,83 a	8,16 a	9 a
20 July	50 cm(4 piece/m²)	2,66 e	7 b	7,5 d
	34 cm(9 piece/m²)	4,16 d	7,66 a	8 c
	25 cm(16 piece/m²)	5,33 c	8,16 a	8,3 b
20 August	50 cm(4 piece/m²)	1,5 g	2,5 e	2,5 g
	34 cm(9 piece/m²)	2 f	3 de	3 f
	25 cm(16 piece/m²)	2,5 e	3,5 d	3,5 e

CONCLUSION

In this study, it was tried to determine the optimum seedling planting distance and time in the vegetative turfgrass establishment with turfgrass seedlings in the "Meyer" variety of *Zoysia japonica* species. According to the results of this research, the highest quality and dense grass cover was obtained in the shortest time, 8 weeks after planting in parcels planted at 25 cm (16 pieces / m²) intervals in June. As the number of seedlings planted per unit area increases, the planting cost per square meter increases. However, if *Zoysia japonica* 'Meyer' seedlings are planted in the early summer (June), an area with high quality and 100% grass cover can still be created with wide planting distances before the growing season ends. In Mediterranean conditions, if planting is to be done in July, it may be recommended to increase the planting density, preferably to apply 25 cm planting distance. In August, a sufficient turfgrass cover (71%) could not be achieved at the end of the same growing season even at 25 cm planting distance. Therefore, it is not recommended to plant 'Meyer' variety in August under these trial conditions.

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CHAPTER 9

WEED MANAGEMENT IN ORNAMENTAL PLANTS

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INTRODUCTION

Weeds are a problem in areas where ornamental plants are grown. Recognition of these weeds is important in determining the appropriate control method with them. The area where ornamental plants will be planted must first be a place without foreign weeds. If an area with weeds is to be selected, planting should be performed after the tillage and herbicide application. Since ornamental plants contain many plant species with grass and broadleaf, maintenance operations such as fertilization and irrigation should be performed more precisely in order to prevent the contamination of weeds in these areas. Otherwise, difficulties will be experienced in the fight after contamination due to the fact that weeds can have both grass and broadleaf.

Weeds can be kept under pressure by using mulching and cover crops in ornamental plants. Some weeds can control with picking or hoeing. If manual collection or hoeing is to be performed, it will be easier to perform these operations when the soil reaches the proper consistency as the soil will soften after rain or irrigation. One-year weeds are easier to control with hoeing. Managing them before setting seeds significantly reduces their density. However, herbicides can also be preferred in terms of practicality and cheapness. However, herbicides to be used in ornamental plant production areas should be carefully selected. Label information should be read carefully and used according to the information here. It can be said that especially annual ornamental plants are more sensitive to herbicides. In suitable ornamental plants, the appropriate herbicide should be applied at the appropriate dose and at the appropriate time.

Soil herbicides should be preferred to suppress annual weeds. For the control of perennial weeds, post-emergence herbicides should be applied when these weeds are 20-25 cm and actively grow. Perennial weeds can be suppressed with 3 years of regular herbicide use. Weeds are still an important problem in ornamental plants, and there is a need to develop alternative soil disinfection with steam and cover crops, also modern methods such as robotics and drone technologies.

1. ORNAMENTAL PLANTS DESIGN

The most important approach in ornamental plants and landscapes are the planning and preparation phases (Savé, 2009). Choosing the suitable ornamental plant and suitable soil for the ornamentals is important for a successful preliminary (Gülser et al., 2011; PennState, 2020). A great advantage of ornamental plants is that they can produce in vitro and contain species suitable for many different soil structures, from dry soils to wet soils (Çığ & Başdogan, 2015; Stack, 2016). However, in the cultivation of ornamental plants, weeds can be a problem for many years, from planting / before planting to every stage of development (PennState, 2020). Weeds compete with plants for water, mineral matter, light, and place (Mennan et al., 2020).

Planting ornamental plants in a certain place of landscape will provide more comfortable weed control in other parts of areas (Papatya Seckin, 2018). Weed control becomes difficult in an ornamental plant area established with a mixture of grass, sedge, broadleaf, tuberous, bulbous, rhizome and stolon ornamental plants (Norcini & Marble, 2020). Preventive and cultural measures in ornamental plants and landscape are necessary for sustainable weed management (AgriLife, 2020). As a cultural measure, dense planting, the use of mulches and cover crops give results as much as herbicide use (Mennan et al., 2020). In the use of cover crops, those that cover the soil and allelopathic to weeds should be selected (McCurdy & Denny, 2018).

Irrigation is an important agricultural process in ornamental plants (Cirillo et al., 2013). Drip, sprinkler and flood irrigation can be performed in ornamental plant areas. The transportation of weed seeds is more in flood irrigation. Planting should be performed taking into account the flow or accumulation of rainwater. It is known that weed seeds overflow with rainwater (Saha et al., 2019). Regulations for efficient use of rainwater for irrigation water will increase efficiency (Singh et al., 2020).

It is important to benefit from water while making landscape arrangements, but some situations should not be ignored:

a) Flood irrigation or rainwater may increase the risk of contamination of weed seeds,

b) Puddles can create a suitable environment for aquatic weeds to thrive. Special weed control techniques such as partial burning or total herbicide application should be applied to areas with such problems (McGiffen et al, 2014). Whether it is rainwater or irrigation water, proper drainage should be provided. It should be designed in a way that allows the soil and the plant to receive as much water as they need. Soil change may also be considered in small areas that hold excess water and where weeds cannot be controlled in landscapes (PennState, 2020).

2. COMMON WEEDS

Weeds are self-growing and undesirable plants in ornamental plants (Radosevich et al., 1997). Weeds are an important problem in ornamental plants and cannot be ignored (Martins et al., 2019).Weeds in ornamental plants, landscapes and turfgrass differ from those of orchards, vegetables and field crops (Saha et al., 2020). In ornamental plants, soil transplantation and mulching are the main factors affecting the change in weed species and density (Iqbal et al., 2020). Weeds that are a problem in ornamental plants can be divided into two categories: generative, seed-propagating, and vegetative, that is, rhizome-propagating (Foo et al., 2011). Table 1 lists some of the most troublesome weeds.

Annual weeds can be controlled more easily with the use of preemergence or preplant herbicides, in addition to hand picking and hoeing. (Neal, 2020). Perennial weeds are more difficult to control, for this post-emergence herbicide use and mechanical control are used for this purpose (Ruedo-Ayala et al., 2010). *Cyperus esculentus, C. rotundus, Eleusine indica,* and *Stellaria media* are troublesome weeds in ornamentals as seen in Table 1.

Table 1: Troublesome ornamental landscape weeds (McCurdy & Denny, 2018)

Scientific names	Common names	Life cycles
Amaranthus spp.	Pigweed	Annual
Brachiaria platyphylla	Broadleaf-signalgrass	Annual
Commelina diffusa	Spreading dayflower	Annual
Cynodon spp.	Bermudagrass	Perennial
Cyperus spp.	Annual sedges	Annual
Cyperus spp.	Nutsedge	Perennial
Digitaria spp.	Crabgrass	Annual
Diodia virginiana	Virginia buttonweed	Annual
Echinochloa spp.	Barnyardgrass	Annual
Eleusine indica	Goosegrass	Annual
Hydrocotyle spp.	Dollarweed	Perennial
Kyllinga brevifolia	Green kyllinga	Perennial
Kyllinga gracillima	False-green kyllinga	Perennial
Kyllinga sesquiflorus	Annual kyllinga	Annual
Kyllinga squamata	Cock's comb kyllinga	Annual
Murdannia nudiflora	Doveweed	Annual
Oxalis spp.	Woodsorrel	Annual
Phyllanthus urinaria	Chamberbitter	Annual
Poa annua	Annual bluegrass	Annual
Setaria parviflora	Knotroot foxtail	Perennial
Setaria spp.	Foxtail	Annual
Smilax spp.	Greenbrier	Perennial
Stachys floridana	Betony	Perennial
Stellaria media	Chickweed	Annual

3. CULTURAL WEED CONTROL

Improper irrigation is most frequently at fault for weedy ornamental beds. When possible, landscape managers should irrigate based upon plant demand rather than using daily or weekly irrigation timers (Garcia-Navarro et al., 2004). In most ornamental beds, there are intense flashes of weed emergence during the warm summer months when moisture is excessive (after rainfall or when landscapes are irrigated too much or too frequently).

Mulching materials may help control weeds in ornamental landscapes (Chalker-Scott, 2007). When plant canopy is lacking, mulch may help prevent seedling establishment. However, when overapplied, mulch may compromise the health of desired ornamentals. A mulch layer of 1 to 2 inches, and no more than 3 inches, is generally recommended. Apply mulch evenly around the base of shrubs and trees, and do not create mounds or "mulch volcanoes". Cloth weed barriers are relatively cheap, but they are rarely used during initial installations around the home and commercial landscapes.

In some instances where new landscape beds are installed or old beds are being redesigned, this option makes sense, as long as these areas will not contain living ground covers or annuals. Practices such as planting ornamental plants in clusters and planting grass plants in open areas can suppress weeds. As a cultural practice, tactics such as solarization and soil disinfection with steam are not very common in ornamental plants (Stapleton, 2000; Fennimore et al., 2014).

4. MECHANICAL WEED CONTROL

There is no substitute for hand-weeding of landscape beds. Complete chemical and cultural controls of weeds are not possible. When hand-removing weeds, it's important to excavate or extract roots because many plants have the ability to regrow from underground roots and stems. Applying mulch afterward may help suppress regrowing weeds (van Donk et al., 2011).

Mowing and tillage are the most widely used mechanical methods (Pellegrini et al., 2016). If necessary, deep plowing with plow may reduce the weed density that will germinate that year. It is known that sensitive sensor robotic and drone technology is not widely used in ornamental plants.

5. CHEMICAL WEED CONTROL

5.1. Preemergence Herbicides

Preemergence herbicides prevent seedling establishment (Chen et al., 2013). They must be applied preventively before major weed emergence. Because preemergence herbicides degrade over time, it is necessary to reapply them for adequate year-round coverage. Typical application dates are based on the emergence cycles of common landscape weeds. Apply in the spring before crabgrass emergence when soil temperatures at a 1-inch depth are below 13°C. Apply a second late-spring/early-summer application roughly 6 to 10 weeks later.

Recommendations differ for each product, so always read the label. Preemergence herbicides are formulated as either liquid or granular products (PPP, 2020). Each formulation has some distinct advantages. Liquid applications tend to saturate thick mulch layers and penetrate to the soil layer. Granular products may be able to penetrate dense plant canopies; however, these products can lodge in the sensitive whorls of desired plants.

5.2. Postemergence Herbicides

Even with preemergence herbicides, some amount of hand-weeding will be required. Selective and nonselective herbicide applications may also be necessary (Martins et al., 2019).

Landscape managers should be properly trained to scout and identify sensitive plant material in order to prevent damage to sensitive ornamentals (Marble et al., 2015).
This is not an exhaustive list and does not imply product recommendations. Always read and follow label directions. When applying any herbicide, make sure to use a sprayer that is thoroughly rinsed and cleaned. The only way to be absolutely sure a sprayer is clean is to avoid using it to apply potentially harmful herbicides (Saha et al., 2017).

5.3. Grass Control

When applied appropriately, certain herbicides control grasses within broadleaf plantings (Thetford et al., 2009). These herbicides are sometimes referred to as ACCase inhibitors. They target a form of the ACCase enzyme that is unique to grass. For instance, sethoxydim and fluazifop control bermudagrass in non-grass plantings.

Table 2: Common preemergence herbicides for ornamental landscapes (McCurdy & Denny, 2018)

Trade name	Active ingredient
Hi-Yield Dimension	Dithiopyr
Surflan, Balan	Oryzaline
Pendulum 2G	Pendimethalin
Pendulum Aquacap	Pendimethalin
Lebanon Treflan 5G	Trifluralin
Harrell's 4.8G	Trifluralin + oxyfluorfen
Freehand	Pendimethalin + dimethenamid
Snapshot	Trifluralin + isoxaben
Gemini	Prodiamine + isoxaben
Showcase	Trifluralin + isoxaben + oxyfluorfen
Goal	Oxyfluorfen
Ronstar, RegalStar	Oxadiazon
SureGuard, BroadStar	Flumioxazin
Goose and Crab	Oxadiazon + bensulide
Tower	Dimethanamid
Devrinol 2G	Napropamide
Gallery	Isoxaben
Casoron 4G, Barrier	Dichlobenil
Specticle G	Indaziflam
Barricade 4FL	Prodiamine

This is not an exhaustive list and should not imply product recommendations. Always read and follow label directions.

Table 3: Common postemergence herbicides for ornamental landscapes (McCurdy & Denny, 2018)

Trade name	Active ingredient	Weeds controlled
Basagran	Bentazone	Used for the control of *Cyperus esculentus* L. and certain broadleaf roots. Groundcover, *Hedera helix*, *Liriope spicata* and *Pachysandra terminalis*.
Envoy	Clethodim	Controls a broad spectrum of actively growing grasses. Does not control broadleaf weeds or sedges. Can be used over-the-top of broadleaf ornamentals. It is effective in controlling a wide range of actively growing lawns. Broadleaf weeds and *Carex* spp. does not check it out. It can be used in large ornamental plants.
Reward	Diquat	Provides control for many weeds that have emerged. It does not control perennial weeds well. It makes visual control of weeds very quickly. Do not spray in contact with actively growing leaves of desired plants.
Fusilade	Fluazifop	It is effective in controlling a wide range of actively growing lawns. It does not control broadleaf weeds and *Carex* spp. It can be used on top for broad-leaved ornamental plants.
Finale	Glufosinate	Provides control for many weeds that have emerged. It does not control perennial weeds well. Do not spray in contact with actively growing leaves of desired plants.
RoundUp	Glyphosate	Various formulations are available. It is effective in controlling a wide range of actively growing lawns. Control can take 10-14 days but provides excellent control for perennial weed. It does not provide pre-exit control. Do not spray in contact with actively growing leaves of desired plants.
Manage	Halosulfuron	*Cyperus spp.* also provides excellent protection. Direct *Cyperus spp.* Without contact with desired ornamental plants. It should be sprayed.
Vantage	Sethoxydim	Provides control for many actively growing lawns. *Broadleaf* weed and *Cyperus spp.* does not provide control. It can be used over broad-leaved ornamental plants.

5.4. Broadleaf Weed Control

Most ornamental plantings contain broadleaf plants. Herbicides that commonly control broadleaf weeds, such as 2,4-D and dicamba, are not safe on broadleaf ornamentals (McElroy & Martins, 2013; PPP, 2020). However, some of these herbicides may have labels that allow selective application as long as there is no contact with foliage, bark, or roots of desired plant material. There are several popular postemergence herbicides (diquat, dimethenamid-P, flumioxazin, glufosinate, glyphosate, indaziflam, oxadiazon, pelargonic acid, sulfentrazone, and sulfosulfuron) with labeling for various weeds within certain nurseries, ornamentals and landscapes. Always use caution because all of these herbicides have the potential to be volatile and/or taken up by roots (Saha et al., 2017).

5.5. Non-Selective Herbicides

Herbicides such as Round-up (glyphosate), Reward (diquat), and several other similar products cause injury and death of most ornamental weeds and landscape plants (Saha, 2017). Therefore, they should be used sparingly and only when applied selectively to undesirable weeds. In addition, the environmental and public health effects of total herbicides such as glyphosate should not be ignored (Pala & Mennan, 2019)

CONCLUSION

Preventive measures, cultural and mechanical control should be prioritized, alternative and modern methods should be researched, and finally, herbicide should be used against weeds in ornamentals. It may be good practices to choose weed-free, to use weed manure to prevent weed contamination, and to carefully carry out maintenance operations such as drip irrigation. Mulching, solarization, steam soil disinfection, and the use of cover crops have become popular in recent years. As the last weed control method, herbicides can be partially used. In the control of annual weeds, soil herbicides should be preferred, also green parts spraying should be preferred for perennials. Herbicides to be applied postemergence should be applied during active growth periods of weeds. Selective and systemic herbicides should be preferred primarily. Non-selective herbicides can be used since the plant diversity is very high in ornamental plants and the licensed selective herbicides are low. However, to avoid phytotoxic effects of total herbicides on ornamental plants, their contact with their green parts should be avoided.

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CHAPTER 10

CONTROL OF *MYZUS PERSICAE* AND *TRIALEURODES VAPORARIORUM* IN *CHRYSANTHEMUM*

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INTRODUCTION

The ornamental plant cultivation presents precious potentiality in agricultural farming, landscape gardening, and in other resembling fields. To achieve good ornamental plant production it is necessary to provide good preconditions like convenient climate, geothermal resources and a qualified workforce (Glavendekić, 2013). Over the past two decades, ornamental plant production has been increased as a result of the growing demand for such plants. In addition, production in indoor places enabled an improvement in the quality and growing plants during a year.

However, those conditions cause certain problems (Balaž et al., 2013). One of the most important problems is the increased occurrence of pests and pathogens. The environment such as greenhouses and other indoor places is highly suitable for the development of the pests population. Those are a very unique space that provides not only a great environment for plants growing but also for the development of plant pests and diseases (Baker & Linderman, 1979). Temperature and humidity in indoor places create favorable conditions for the increasing pest population. The amount of decorative plants is continuously increasing with the importation of the reproductive and planting material including seedling trees, groundcovers, grasses, vines and aquatic plants, bulbs and seeds (Hulme et al., 2018). At the same time, all of this represents the dominant pathway for the introduction of invasive alien plants worldwide (Jiang et al., 2011; Dodd et al., 2015).

Agricultural pests which appear in greenhouses are very well adapted to the living condition. Due to this, the pest population can be controlled only by the implementation of integrated plant protection management. The most common and economically most harmful organisms in the production of ornamental plants in the protected area (greenhouses, hothouses, institutions, apartments) are butterflies (*Trialeurodes vaporariorum* Westw., etc.), aphids (*Aphis gossypii* Glover, *Aphis nasturtii* Kalt., *Aulacorthum solani* Kalt., *Myzus persicae* Sulz., *Macrosiphum euphorbiae* Thom., etc.), California flower thrips (*Frankliniella occidentalis* Pergan.), leaf miners and flies (Diptera: Agromyzidae, Sciaridae) mites (Acari: Tetranychidae, Eriophyidae), nematodes (*Meloidogyne* spp.), snails, moles, etc (Kereši et al., 2016). Aphids are one of the most common plants feeding insects. These damaging pests attack a significant number of crops as a food source, including ornamental plants as well. Aphididae can attack crops in the field as well as in greenhouses. *M. persicae* appears on decorative plants including carnations, chrysanthemums, poinsettia and different kind of roses (Petaković & Glavendekić, 2013). Large populations of aphids may reduce the aesthetic quality of plants and even completely damage small plants such as annual flowers, causing wilting, yellowing, and curling of leaves. Great populations of *M. persicae* can attack young shoots, which causes the stressful condition, reflected in withering and losing growth. Aphids feed by piercing plant tissue and sucking the host's sap but the most damage is caused by transmitting the plant viruses. According to some experts, peach aphids are the greatest vectors of viruses.

The protection of ornamental plants includes biological, mechanical and chemical measures. The use of beneficial organisms, such as predators and parasites, helps to protect ornamental plants (Lecomte et al., 2016), especially in home gardens. However, their use can not keep a large population of pests below damaging levels. Mechanical measures, like picking or knocking off the insects of the plants could be an effective measure in the small gardens around the house, while it is not efficient enough on large fields or greenhouses.

This situation often requires the intensive application of pesticides, and as a consequence, the endangerment of greenhouse workers (Li & LaMondia, 2010) and a negative impact on the environment. Nowadays, to avoid this, integrated pest management (IPM) should be applied. IPM is very important for floriculture and nursery crop production, including sanitation, clean stock, host resistance, and control through biological, cultural, environmental, chemical, and regulatory means. Integrated pest management represents the use of all available strategies to manage pests - biological and mechanical measures, and as a final solution, pesticide application. Recently, new biocontrol and chemical products improve the control of pests, while meeting the requirement for minimal environmental impact. The development of new crops, as well as production technologies, provides opportunities for new pests, and due to this, new possibilities for the control of these pests are required (Daughtrey & Benson, 2005).

1. CHRYSANTHEMUMS

Chrysanthemum is one of the most beautiful and economically the most important ornamental plants all over the world. The genus *Chrysanthemum* includes more than 100 species. It is a shrubby plant from the Asteraceae family, originating from Asia and Northeast Europe. Chrysanthemum flowers are used for decoration (Mladenović et al., 2016). Among a large number of species, *Chrysanthemum sinense* and *Chrysantemum indicum* are commercially the most important. They are available in a number of different varieties and a wide range of colors. Some varieties are susceptible to low temperatures, requiring greenhouses conditions, however, a number of perennial varieties that can be grown in a field conditions. As ornamental plants, those were firstly grown in China. It is estimated that there are over 20,000 cultivars in the world, with 7,000 located in China. Besides China, the largest growers of chrysanthemum are the Netherlands, Colombia and South Africa.

Apart from its decorative character, the dried heads flower of *Chrysanthemum cinerariefollium* are used as a raw material for the extraction of pyrethrin insecticides. Pyrethrins are the common name for six esters (pyrethrin I, pyrethrin II, cinerin I, cinerin II, jasmoline I, jasmoline II) with insecticidal activities. As insecticides, they are used in the control of aphids, caterpillars, thrips and moths in different crops. Pyrethrins belong to broad-spectrum insecticides with toxicity to beneficial species. However, due to the fact that they briefly retain insecticidal properties on the leaf surface, biodegradability and photolability, their negative impact is insignificant. Pyrethrins act on the insect's nervous system, and in sublethal doses, they act as insect repellents. They are harmful to fish, and less toxic to mammals and birds than many synthetic insecticides.

Chrysanthemums are propagated by cuttings. For late-flowering, they are planted in indoor conditions. For use of flowres, the production of chrysanthemums requires rich soil, deeply plowed and loose. Feeding should be based on the results of soil analyzes. After planting, chrysanthemums require 3-6 weeks of a long day to form a stem of sufficient length. Too dense distribution contributes to the increased appearance of the disease or elongation of the plant stem, as well as the reduction of the formation of new buds. There are several limiting factors in the production of chrysanthemums; primarily those are diseases and pests. The most common fungal diseases causing agents are *Pythium* sp., *Phytophtora* sp., *Ryzoctonia* sp., *Fusarium oxysporum* f. sp. *Chrysanthemi*, *Verticillium dahlia*, *Septoria chrysanthemi*, *Cercospora chrysanthemi*, *Botrytis cinerea*, *Puccinia chrysanthemi* and etc. The most significant of the viral diseases are Chrysanthemum B virus, Tomato aspermy virus on chrysanthemum and Chrysanthemum stunt viroid. The most common pests are those usually found in greenhouses, such as aphids, with the most important species *Myzus* *persicae*, *Trialeurodes vaporariorum*, *Macrosiphoniella sanborni*, Trips sp. and etc. (Kereši et al., 2016).

2. EVALUATION OF THE EFFICACY OF INSECTICIDES IN THE CONTROL OF *MYZUS PERSICAE* AND *TRIALEURODES VAPORARIORUM*

In order to evaluate the efficacy of alpha-cypermethrin, bifenthrin and chlorpyrifos in the control of *Myzus persicae* and *Trialeurodes vaporariorum* the trials were carried out during 2018 in accordance with the standardized OEPP methods (EPPO PP 1/23(2); EPPO PP 1/152(4); EPPO PP 1/135(4)) in Multiflora cultivar chrysanthemum greenhouse production. Field experiments were carried out in the locality Budisava (Vojvodina, Republic of Serbia). The plant protection products based on alpha-cypermethrin (100 g a.i./L, EC), bifenthrin (100 g a.i./L, EC) and chlorpyrifos + bifenthrin (400 + 20 g a.i./L, EC), were applied at a concentration of 0.025%, 0.05% and 0.1%, respectively. The abovementioned insecticides were foliar applied, by a backpack sprayer with water consumption of 500 L/ha, when chrysanthemums were in the flowering stage.

The experiment was set in four replications in a randomized block design. The number of flying forms of *M. persicae* was estimated at 10 previously marked offsprings per replication and for *T. vaporariorum* the number of live adults and larvae per 1 leaf/plant, per 10 plants in each replication. The assessment was derived on four occasions, which are: just before the treatment, and after 2, 8 and 15 days of treatment. The efficacy was expressed by Henderson & Tilton (Wentzel, 1963)

and the significance of differences was established using ANOVA for a confidence interval of 95%.
Among the tested insecticides, on the list of approved active substances for use in plant protection in 2020 is only the active substance alpha-cypermethrin, while insecticides bifenthrin and chlorpyrifos were not approved (EU Pesticides Database, 2020).

According to the aphids abundance that was evaluated prior to setting up the experiment (Table 1) the average abundance of *M. persicae* ranged from 133.7 to 170.2 in different variants. Two days after the treatment of the above-mentioned insecticides, the abundances of *M. persicae* were significantly lower compared to the control treatment. The efficacy of the insecticides ranged from 92.8 up to 95.9% . These values are at the same significance level.

Table 1: Average number of green peach aphids (*M. persicae*) and insecticide efficacy

Insecticides	before the treatment		2 days after the treatment		
	$\bar{x}$	±Sd	$\bar{x}$	±Sd	E%
alpha-cypermethrin	170.2 a	21.5	12.2 b	4.03	93.9
bifenthrin	137.2 a	25.3	11.5 b	2.61	92.8
chlorpyrifos + bifenthrin	161.7 a	38.7	7.75 b	2.50	95.9
control	133.7 a	24.9	157.5 a	11.3	
LSD 5%	42.11*		10.14**		

E%- efficacy; *F=0.97; p<0.45;**F=542.2; p<0.01

Eight days post-treatment the number of Aphids was notably lower when compared to the control one. For the insecticides used in this trial, the efficacy ranged from 94.5% to 96.4%. Fifteen days after the treatment, the number of *M. persicae* was significantly lower compared to the control treatment. The results of the plant protection products efficiency reported in Table 2 indicate that efficiency was still high and in a range from 93.2% to 95.2% (Vuković et al., 2019).

Table 2: Average number of green peach aphids (*M. persicae*) and insecticide efficacy

Insecticides	8 days after the treatment			15 days after the treatment		
	$\bar{x}$	±Sd	E%	$\bar{x}$	±Sd	E%
alpha-cypermethrin	7.50 b	2.65	96.8	12.2 b	4.57	95,2
bifenthrin	6.75 b	1.71	96.4	10.5 b	5.92	95.0
chlorpyrifos + bifenthrin	12.5 b	1.29	94.5	13.7 b	4.03	94.3
control	189.5 a	32.2		199.5 a	8.35	
LSD 5%	26.04*			9.39**		

E%- efficacy; *F=122.2; p<0.01; **F=110.2; p<0.01

M. persicae can develop two different ways of resistance. The most usual ways are increasing the insecticide detoxification and change of site sensitivity. Due to this, resistance may be developed by a large number of insecticides that have a different mode of action. The lower sensitivity of *M. persicae* is developed to the most used group of insecticides which means that the resistance has been developed to some degree, but the studied colonies of green peach aphids in Serbia do not belong to the group of high resistant Aphids (Vučetić et al., 2007).

As a consequence of the increasing use of insecticides, green peach aphids have developed resistance to dozens of insecticides like neonicotinoids (imidacloprid), organophosphates (chlorpyrifos), carbamates (carbaryl and thiodicarb), and synthetic pyrethroids (deltamethrin) in numerous countries (Slater et al., 2012: Bass et al., 2014; Puinean et al., 2013).

Tang et al. (2017) have studied the sensitivity of 11 populations of *M. persicae* with the aim of assessing the resistance of seven different insecticides. The colonies were collected from the different sites of China. The results of the study showed that *M. persicae* population had been developed several levels of resistance to each insecticide that was analyzed. The field population developed high levels of resistance to beta-cypermethrin and cypermethrin. The resistance to bifenthrin was low. Seven out of eleven tested populations of *M. persicae* showed resistance to bifenthrin (12-38 times). According to Umina et al. (2014), *M. persicae* in Australia developed a moderate level of resistance to organophosphate insecticides and a high level to alpha-cypermethrin. Similar results in Australia, with the development of resistance to organophosphates, were reported by Edwards et al. (2008). The same study reported resistance to synthetic pyrethroids in the range from 25% to 100% of sampled aphids (Edwards et al., 2008). High developed resistance to synthetic pyrethroids has been reported over different parts of Europe, caused by kdr and super-kdr mutations (Nauen & Elbert, 2003). With the aim to minimize the consumption of synthetic pesticides due to their harmful effects on the environment, leaves extracts of *Artemisia herba-alba*, *Eucalyptus camaldulensis* and *Rosmarinus officinalis* were studied in the laboratory for insecticidal effect against *M. persicae*. It is established that etheric plant extracts were very effective and caused high mortality which suggests that these compounds can be used in bioinsecticides for integrated pest management (Nia et al., 2015).

Table 3: Average number of adults greenhouse whitefly (*T. vaporariorum*) and insecticide efficacy

Insecticides	before the treatment		2 days after the treatment		
	$\bar{x}$	±Sd	$\bar{x}$	±Sd	E%
alpha-cypermethrin	9.5 a	3.11	1.75 b	0.60	83.0
bifenthrin	9.75 a	4.03	1.25 b	1.49	88.1
chlorpyrifos + bifenthrin	11.5 a	6.45	1.5 b	0.58	87.9
control	12.7 a	3.50	13.75 a	4.57	
LSD 5%	7.63*		4.25**		

E%- efficacy; *F=0.41; p<0.75; **F=21.3; p<0.01

Results of the analysis of the efficacy of different insecticides in the control of greenhouse whitefly are shown in Tables 3-6. Two days after treatment, the number of greenhouse whitefly adults was at a significantly lower level compared to the control, and the efficiency of the applied insecticides was 83.0-88.1% (Table 3).

Eight days after the application the number of *T. vaporarium* was on a notably lower level compared to the control. The efficacy of the tested insecticides was 87.2-91.2%. The number of greenhouse whitefly 15 days from the application of the insecticides is 82.0-92.4% (Table 4).

Immediately before treatment, the number of greenhouse whitefly larvae was at the same level of significance in all variants, while two days after insecticide application (Table 5).

Table 4: Average number of adults greenhouse whitefly (*T. vaporariorum*) and insecticide efficacy

Insecticides	8 days after the treatment			15 days after the treatment		
	$\bar{x}$	±Sd	E%	$\bar{x}$	±Sd	E%
alpha-cipermethrin	1.5 b	0.50	87.2	1.25 b	1.29	88.5
bifenthrin	2.0 b	0.81	83.4	2.0 b	0.81	82.0
chlorpyrifos + bifenthrin	1.25 b	1.25	91.2	1.0 b	1.41	92.4
control	15.7 a	3.30		14.5 a	3.11	
LSD 5%	2.36*			2.89**		

E%- efficacy; *F=77.8; p<0.01; **F=56.1; p<0.01

Table 5: Average number of larvae greenhouse whitefly (*T. vaporariorum*) and insecticides efficacy

Insecticides	before the treatment		2 days after the treatment		
	$\bar{x}$	±Sd	$\bar{x}$	±Sd	E%
alpha-cypermethrin	18.5 a	3.11	1.25 b	0.95	94.2
bifenthrin	10.7 a	7.05	1.5 b	1.29	88.0
chlorpyrifos + bifenthrin	10.2 a	3.09	1.0 b	1.41	91.6
control	13.0 a	4.54	15.2 a	4.99	
LSD 5%	7.72*		4.53**		

E%- efficacy; *F=2.0; p<0.18; **F=24.5; p<0.01

Eight days after the application the number of *T. vaporarium* larvae was on a significantly lower level compared to the control (Table 6). The efficacy of the tested insecticides was 90.1-94.2%. The number of greenhouse whitefly 15 days after the treatment is significantly lower compared to the control. The efficacy was still high and ranged from 85.4-95%.

Based on the results of Erdogan et al. (2012), populations of *T. vaporarium* showed different levels of resistance to chlorpyrifos, while resistance factors were ranged between 7.2 and 12.9 times.

Table 6: Average number of larvae greenhouse whitefly (*T. vaporariorum*) and insecticides efficacy

Insecticides	8 days after the treatment			15 days after the treatment		
	$\bar{x}$	±Sd	E%	$\bar{x}$	±Sd	E%
alpha-cypermethrin	1.5 b	1.29	93.4	1.0 b	2.49	94.4
bifenthrin	0.75 b	1.48	94.2	1.5 b	2.38	85.4
chlorpyrifos + bifenthrin	1.25 b	1.49	90.1	0.5 b	0.99	95.0
control	16.0 a	4.16		12.5 a	4.79	
LSD 5%	3.58*			4.96*		

E%- efficacy; *F=43.8; p<0.01; **F=14.9; p<0.01

Also, the susceptibility of three populations of *T. vaporarium* to bifenthrin insecticide was examined, and each showed significant resistance (up to 662-fold) compared to the susceptible population (Karatolos et al., 2012). In Greece, Kapantaidaki et al. (2018) evaluated the susceptibility of 35 populations of greenhouse whitefly to imidacloprid, bifenthrin, and spiromesifen, and different resistance factors (207, 4657, and 59 times, respectively) were obtained. In contrast to the previously mentioned studies, the population of *T. vaporarium* in our experiment showed significant sensitivity to the applied insecticides. Only differences were found in the sensitivity of different development stages, i.e. the larvae were more sensitive than the adults.

Since 2019, the insecticide flupyradifuron from the chemical group of butenolide has been used in the control of plant aphids and *T. vaporarium* in ornamental plants in the Republic of Serbia. Butenolides act as an agonist of nicotinic acetylcholine receptors. Flupiradifuron is a systemic insecticide, with contact and digestive effect on all stages of sucking insects. Feeding of pest stops quickly and it is of great importance for the transmission of the virus from the insect vector to the cultivated plant.

CONCLUSIONS

The number of insecticides with different modes of action for the control of economically important pests, especially aphids, in ornamental plants is very limited. Due to this, the implementation of an anti-resistance strategy is complicated, and the application of the available active substances has to be controlled. Finally, integrated pest management, including non-chemical measures, should be the basis on the protection of ornamental plants.

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CHAPTER 11

ENSURING SUSTAINABILITY IN HEAVY METAL CONTAINED SOIL AND WATER RESOURCES BY CULTIVATING ORNAMENTAL PLANTS

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INTRODUCTION

It is a known fact that environmental pollution seriously threatens the world today. Although various measures are tried to be prevented, environmental pollution is increasing day by day in parallel with the rapid population growth and the development of industry and technology in agriculture and other fields. Especially towards the middle of the twentieth century, with the transition to modern agriculture, the increase in the use of chemical inputs in aquaculture caused the destruction of soil and water resources (Akın, 2009). Soil and water are indispensable elements for living creatures to survive. In the periods when the industry was not yet developed, there was not much scientific knowledge, modern agriculture was not done, and the population was low, there was no contamination in the soil and water resources as in other environmental elements. However, as a result of increasing industrial activities, urbanization, and rapid population growth with the industrial revolution, soil and water pollution has emerged as a serious environmental problem. In line with this increasing demand and pollution, the quality and quantity of soil and water resources have decreased. Major pollutants in soil and water are: acids and alkalis, detergents, household waste and fertilizers, food industry waste, various gases, heat, various heavy metals, nutrients, oils and dispersants, organic toxic waste, pathogens and pesticides (Gana & Toba, 2015; Menteşe, 2017).

Heavy metals contaminate especially soil, water, and air due to their various usage areas. Due to its toxic effect even at very low concentrations and its long biological half-life, heavy metals that come into question in environmental pollution harm living things if they are found above a certain level (Okçu et al., 2009).

The most important effect of heavy metal pollution in soil and water resources on humans is that it causes various diseases. Because the pollutants that grow on the contaminated soil or get into the plants that are irrigated with polluted water, pass into the body of the people who feed on these plants and cause negative effects on their health. Therefore, the protection of natural resources and sustainable natural resource management for future life and generations are very important for the protection of ecosystems and biodiversity (Moiseenko et al., 2012).

In addition to preventing this increasing environmental pollution, the recovery of destroyed soil and water resources is necessary. Various methods are developed to reduce the pollution caused by the unconscious consumption of agricultural land and freshwater resources. Some of these methods are; Various remedial methods such as purification of water resources, isolation and immobilization applications, soil washing, the use of organic materials as soil improvers, phytoremediation, and microorganism applications are used (Yassaoğlu et al., 1987).

Various studies have determined that pollutants in soil and water resources adversely affect plant growth. For example, it has been determined that vegetables, fruits, and field crops (wheat, corn) grown in heavy metal-containing soils have negative consequences in their yield and quality, on the other hand, the health of people and animals consuming these plants is adversely affected and causes permanent diseases. By choosing ornamental plants that are resistant to heavy metals and other pollutants in these polluted soil and water resources, which are not directly included in the food chain, these polluted soil and water resources can be improved and recovered (Assuncao et al., 2003).

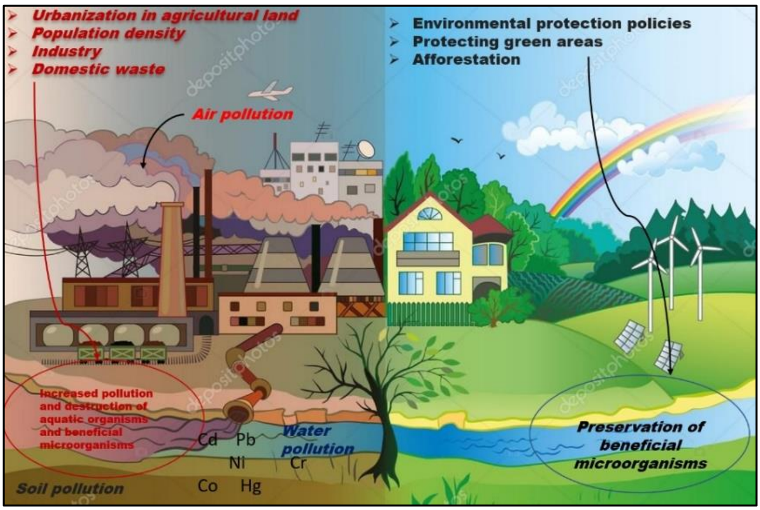


Figure 1: Environmental pollution overview

In recent years, it is aimed to identify plants resistant to heavy metals and to increase the policies for their use. They are grouped under different groups as ornamental plants, outdoor ornamental plants, indoor ornamental plants, and cut flowers. These plants; Apart from beautifying the area where they are used, are of great importance in terms of social, cultural, human, and environmental health and tourism. The development of environmental awareness in the world in recent years has led to an increase in park and garden arrangements and an increase in the demand for indoor and outdoor ornamental plants. The production of these needed ornamental plants using contaminated soil and water resources will be an important source of income for farmers. Until now, many plants have been used in remediation, but there is little information available about the use of ornamental plants to improve contaminated soil and water resources (Liu et al., 2008; Watharkar et al., 2013).

Table 1: Maximum heavy metal concentrations required in waste (Şengül et al., 1986)

Heavy metal (polluting)	*Maximum concentration limit*
Zn (Zinc)	2000 (mg/kg)
Cu (Copper)	1000 (mg/kg)
Ni (Nickel)	200 (mg/kg)
Cd (Cadmium)	15 (mg/kg)
Pb (Lead)	1000 (mg/kg)
Hg (Mercury)	10 (mg/kg)
Cr (Chromium)	1000 (mg/kg)
B (Borium)	1000 (mg/kg)

In this compilation study, it is aimed to investigate the effects of environmental pollutants on reducing the negative effects of environmental pollutants on natural resources and ensuring the sustainability of these resources, as well as these versatile effects of ornamental plants, and also this study is intended to be a resource for similar studies to be conducted in the future.

1. HEAVY METALS IN SOIL AND WATER RESOURCES AND HEAVY METAL-PLANT RELATIONSHIP

Heavy metals are elements with a specific gravity of more than 5 g/ cm^3 and an atomic number of more than 20. The term heavy metal has entered the literature with environmental pollution. It is used as a connotation in terms of contamination and toxicity. While some heavy metals such as copper (Cu), zinc (Zn), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), cobalt (Co) are essential micronutrients for plant growth, arsenic (As) Some heavy metals such as mercury (Hg), Cd and Pb are elements that are not necessary for plant growth (Niess, 1999; Özay & Mammadov, 2016).

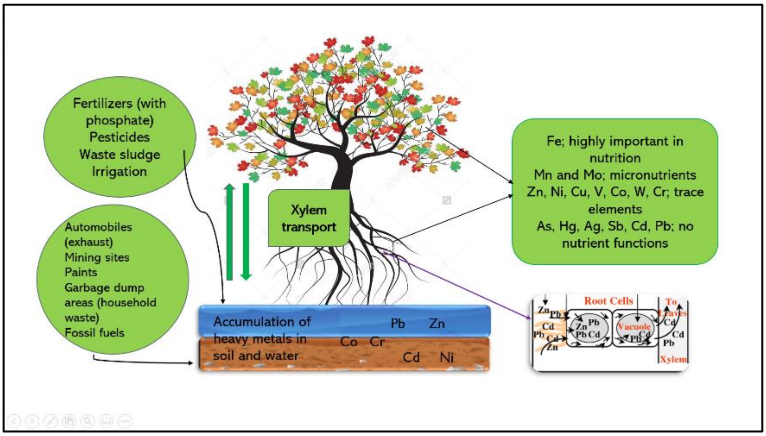


Figure 2: Accumulation and transport of heavy metals in plants

The concentration of heavy metals above a certain level in the atmosphere, water, and soil causes serious problems for all living things. It is known that heavy metals cause stress in plants by directly or indirectly affecting physiological and morphological functions, especially since they exceed certain doses (Sossé et al., 2004). Studies have shown that plants can absorb metals in the form of gas in the air through their stomata and ionic metals are absorbed into the plant by passing through the cuticle layer of the leaves. On the other hand, plants, especially those of terrestrial plants, mostly absorb metals from the soil and irrigation water through their roots. Since effects such as soil and water pH, temperature, the amount of organic matter, the presence of other metals, and microorganisms will change the mental capacity in the soil and water solution, this will affect the metal uptake of the plants. Metal uptake also varies depending on the type of plant. Features such as root cation exchange capacity and root surface area affect metal uptake. Metals taken from the roots are transported to the stem and leaves and other organs through xylem (Marschner, 1995; Greger, 1999; Memon et al., 2005).

The excessive accumulation of heavy metals accumulated in soil and water resources by plants in their tissues and organs negatively affects the development of vegetative and generative organs of plants. Also, many physiological events such as transpiration, stomatal movements, water intake, photosynthesis, enzyme activity, germination, protein synthesis, membrane stability, hormonal balance are negatively affected in plants (Shanker et al., 2005; Benavides et al., 2005).

2. THE USE OF ORNAMENTAL PLANTS IN AREAS CONTAMINATED WITH HEAVY METALS

In the fight against pollution, it is very important to know the rate of pollution in soil and water resources and the factors that cause pollution, to determine the risks it creates, and to know the conditions required for the most appropriate cleaning of soil and water. Especially for the determination of the pollution level of a heavy metal contaminated source and its treatment, high costs, and time is required for using technological and electrochemical methods. For this reason, research has shown that some ornamental plants can be used in remediation and more detailed research has begun to be done on this subject.

2.1. Phytoremediation

Phytoremediation is defined as the process of clearing contaminated areas from contaminants by growing resistant plants. Plants absorb, accumulate, store or break down these pollutants and ensure that contaminated areas are cleaned. Phytoremediation is important because it is a sustainable, cost-effective and environmentally friendly technique compared to other breeding methods. It is very important that the plants used in phytoremediation first survive in these areas contaminated with heavy metals and then recover these pollutants by removing them from the area. Recently, it is aimed to carry out studies to investigate phytoremediation efficiency in various ornamental plants and to determine plant species suitable for this purpose.

Plants generally used in phytoremediation technique are called hyperaccumulative plants. These plants; they are plants that accumulate 50 to 500 times more metal than the metal content in soil and water in their leaf branches and stems. In other words, these plants can contain 100 to 1000 times more organic matter than non-hyperacumulative plants with their organs located above soil and water (Brooks, 1998; Clemens, 2006). For example; Some hyperacumulative plants such as *Thlaspi, Urtica, Taraxacum officinale, Chenopodium, Polygonum aviculare* L. and *Allyssim* have the ability to accumulate heavy metals such as cadmium, copper, lead, nickel and zinc and survive when grown in heavy metal-sourced soil and water resources. Therefore, the cultivation of these plants can be considered as an indirect method of purification of contaminated soil and water resources (EPA, 1995; Raskin et al., 1997; Milner & Kochian, 2008; Yurdakul, 2015).

2.1.1. Some Phytoremediation Methods

2.1.1.1. Phytoextraction

It is the removal of toxic metals from polluted soil by using plants that accumulate metal. The metal ratios that plants take from soil and water are very important. In addition, some plants that grow on wastes called vegetative cover or in polluted areas can be effective in preventing erosion. For example, *Alyssum murale* was identified as the best Cd and Ni collector in a study (Hansruedi, 1997; Yurdakul, 2015).

2.1.1.2. Rhizofiltration

Root zone filtration is the removal of toxic metals from water using plant roots. For example, it is stated that *Brassica juncea* L. Czern and *Helianthus annuus* L. plants are effective in Pb removal, contain the Pb absorbed by the root of the plant, and are important in removing Pb at high concentrations (Viatcheslav et al., 1995). In coastal water contaminated with As, Cd, Cu, Cr, Fe, Mn, Ni, Pb, V and Zn, water hyacinth (*Eichornia crassipes*) was improved by phytoremediation, and Cr, Cd, Pb and As in the roots and sprouts of water hyacinth. It has been reported to accumulate (Agunbiade et al., 2009; Yurdakul, 2015).

2.1.1.3. Phytostabilization

Vegetative fixation is to cover the soil surface with plants in order to reduce the pollution of groundwater and prevent the contact of pollutants with the soil, and prevent damage to the plant by reducing the bioavailability of toxic metals. In some trees, for example, poplar acts as a pump, moving the pollutants in the water upwards with their roots, and ensure the removal of pollutants from groundwater (Boisson et al., 1999; Astier et al., 2014; Yurdakul, 2015).

2.1.1.4. Phytodegradation

Degradation in plants is the process of breaking down organic compounds that plants take up with enzymes due to their metabolic structure. Degradation occurs as a result of intake, transport, metabolic activity, and microbial activities. Pb-containing soils and soils without Pb contamination were tested using sunflower (*Helianthus annuus*), sorghum (*Sorghum bicolor*), and Chinese squash (*Brassica chinensis*), and degradation was observed in soils containing heavy metals (Komives & Gullner, 2005; Hamvumba et al., 2014; Yurdakul, 2015).

2.1.1.5. Phytovolatilization

The plant changes its element structure to be released into the atmosphere by transporting some heavy metals such as Hg and Se in the soil from the soil and water sources to the leaves. For example, in a study, it was reported that some plants such as *Brassica juncea* and *Arabidopsis thaliana* can absorb heavy metals in their bodies and turn them into gas form and release them into the atmosphere (Terzi & Yıldız, 2011). Tree species such as *Populus* and *Salix* are widely used in this technique due to their evaporation properties (Pulford & Watson, 2003; Yurdakul, 2015).

2.2. Biotechnolic Methods

Nowadays, it is very important to minimize the nutritional problems of future generations due to the rapid increase in environmental pollutants, the decrease in suitable soil and water resources, and the increase in the population. For this reason, it is aimed to produce plants resistant to heavy metal stress with the developing technology in recent years and to prevent the possible nutrition problem that may arise in the future. Plant biotechnology has led to a better understanding of the mechanisms related to heavy metal tolerance and accumulation in plants. It is aimed to develop new ways to use genetic engineering to modify plants about metal uptake, transport, and internal retention and to increase phytoremediation efficiency. Following the completion of the *Arabidopsis* genome project, several genes associated with heavy metal homeostasis and accumulation were identified by the genome scanning of many other plants (Dhankher et al., 2002). Understanding the genetics and biochemistry of metal uptake, transport, and deposition phytoremediation It can lead to the development of high capacity transgenic plants (Salt & Kramer, 2000; Baker et al., 2000).

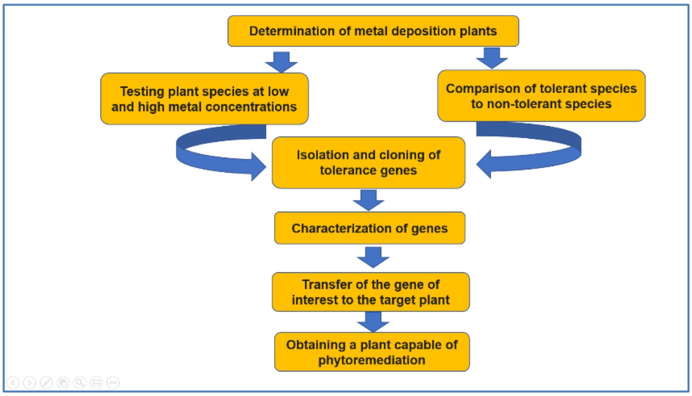


Figure 3: Obtaining plants capable of phytoremediation (Karenlampi et al., 2000)

For example, overproduction of GSH (glutatyon) in *Thlaspi* goesigense plants has been reported to increase tolerance to oxidative stress induced by Cd and Ni. Critical analyzes of the mechanisms of metal uptake, translocation, accumulation, and tolerance in natural metal hyperaccumulator plants will aid in the identification of genes responsible for the synthesis of metal-binding proteins or peptides (Boominathan & Doran, 2003; Freeman & Salt, 2007; Terzi & Yıldız, 2011).

3. SOME STUDIES ON ORNAMENTAL PLANTS AND HEAVY METALS

In a study conducted in India, *Phragmites cummunis* Trin, *Typha angustifolia* L. and *Cyperus esculentus* L. plants were used in the removal of different metals, and the metals accumulated the most by plants, respectively, were Fe>Mn>Zn>Cr>Pb>Cu>Ni>Cd. In addition, it has been determined that *T. angustifolia* L. accumulates zinc in its body rather than roots and leaves (Chandra & Yadav, 2011). In addition, Ahmad et al. (2014) determined that *Phragmites australis* Trin. is one of the plants that holds Zn the most among 11 different heavy metals. It has also been demonstrated that *P. australis* is resistant to high Zn poisons in water.

Phragmites communis Trin.
Source:https://en.wikipedia.org/wiki/Phragmites

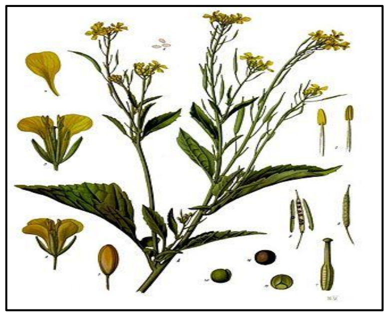
Typha angustifolia L.
Source:https://en.wikipedia.org/wiki/Typha_angustifolia

Cyperus esculentus L.
Source:https://en.wikipedia.org/wiki/Cyperus_esculentus

Salido et al. (2003) used fern *Pteris vittata* L. and *Brassica juncea* L. plants as accumulator plants in a study they conducted to remove arsenic and lead contamination from soils by phytoremediation method. As a result, they revealed that As and Pb pollution in the soil can be prevented by the phytoremediation method.

Toxic metals have negative effects on root growth. The plant's tolerance and soil chemistry determines the plant's tolerance level against heavy metals. Some plants show high tolerance to a metal while others show low tolerance. For example, Cobalt flower (*Crotolaria cobaltica L.*) plant contains 500-800 ppm Co of 1 kg dry matter (Davies et al., 1991).

Pteris vittata L.
Source:https://en.wikipedia.org/wiki/Pteris_vittata



Brassica juncea L.
Source:https://simple.wikipedia.org/wiki/Brassica_juncea

Crotolaria cobaltica L.
Source:https://en.wikipedia.org/wiki/Crotalaria

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<td>Vanlı (2007) examined the reclamation of contaminated soils by phytoremediation method in a study he conducted. For this purpose, phytoremediations were investigated using corn, sunflower and canola plants in soils supplemented with Pb, Cd and B elements. The researcher revealed that these contaminants can be removed from the soil by corn, sunflower and canola plants. He found that the most effective plant in this regard is canola. The phyto-extraction potential of canola plant for heavy metals such as Cd, As, Hg, Pb, Cr, Cu, Zn and Ni was theoretically calculated. In the research, it was stated that there were heavy metal accumulations in the root stem and seed of the plant (Yu et al., 2011).</td>
<td>
Zea mays L.
Source:https://en.wikipedia.org/wiki/Maize#/media/File:ZeaMays.jpg

Helianthus annuus L.
Source:https://tr.wikipedia.org/wiki/Ay%C3%A7i%C3%A7e%C4%9Fi

Brassica napus L.
Source:https://tr.wikipedia.org/wiki/Kanola#/media/Dosya:Brassica_napus_2.jpg</td>
</tr>
</table>

Rai (2010) examined different heavy metal accumulation in the root, above-ground organs, and water samples periodically in his study in the industrial regions of India and *Eichhornia* M. It was found that it accumulates Zn at the highest amount in its aboveground organs compared to other metals, and Zn is the most accumulated metal after Hg in its root. In another study, Zn retention properties of *Eichhornia crassipes* and *Potamogeton crispus* plants were investigated by different techniques. It has been found that *Eichhornia crassipes* is a suitable plant for phytoremediation of Zn due to its rapid mass and rapid growth. It has also been found that the *Potamogeton crispus* plant grows rapidly in freshwater lakes and streams and accumulates high amounts of Zn, Cu, Pb, Mn, Ni, and Hg (Tel-Or & Forni, 2011).	 *Potamogeton crispus* L. Source:https://en.wikipedia.org/wiki/Potamogeton_crispus  *Eichhornia crassipes* Mart. Source:https://en.wikipedia.org/wiki/Eichhornia_crassipes

In their research, the researchers found that Cu accumulated in the shoot and root tissues of the plant in high tests, but that the plants grew in normal order without showing phytotoxicity symptoms at all doses. Flowering is increased; Root and shoot biomass, root lengths and leaf soluble protein remained the same as said control. *Calendula officinalis,* on the other hand, experienced a reduction when they cultivated the L plant under the effect of Cd and Pb mycorrhizal colonization, Cd, and Pb toxicity reduced the editor's skin and also increased the accumulation of Pb and Cd in root and shoot organs. According to this post, it showed that it can be used as a phytoremediation plant containing heavy metal (Borghei et al., 2011; Tabrizi et al., 2015; Goswami & Das, 2016).	 *Calendula officinalis* L. Source:https://en.wikipedia.org/wiki/Calendula_officinalis  *Calendula officinalis* L. Source:https://en.wikipedia.org/wiki/Calendula

Plants such as calla lily (*Zantedeschia aethiopica* L.) is a plant that grows in wetlands contaminated with heavy metal and is widely used in the treatment of wastewater. To evaluate the iron (Fe) tolerance of this plant, it was determined in the study that it can be used as a phytoremediation plant in soils containing Fe (Casierra-Posada et al., 2014). In plants treated with 0-100 µM Pb, they have accumulated Pb in their roots, and this may be the possibility to physically stabilize the Pb table together with the ornamental ones. Lead treatment prevents growth in leaves and higher Pb length (100) and increased malondialdehyde (MDA) condensation. Consequently, *N. oleander* has been proposed as an HMs phytostabilizer (Trigueros et al., 2012; Lajayer et al., 2019).	 *Zaetedeschia aethiopica* L. Source:https://en.wikipedia.org/wiki/Zantedeschia_aethiopica  *Nerium oleander L.* Source:https://en.wikipedia.org/wiki/Nerium

Cd content of the leaves increased by increasing the Cd concentration in the soil. *Gladiolus grandiflorus* L. had the highest tolerance and Cd concentration, but given the plant biomass, the total Cd removal was, respectively, *Chrysanthemum indicum* L. >*Gladiolus grandiflorus* L.>*Tagetes erecta* L. In another study conducted with *Chrysanthemum indicum* L.; A significant decrease in height, root length, and dry biomass was observed at the maximum dose of Pb applied. Pb accumulation trend was: Root> shoot >flower. The results suggested that *C. indicum* could be used for phytoextraction from moderately Pb contaminated soils (Lal et al., 2008; Mani et al., 2015; Lajayer et al., 2019).

Tagetes erecta L.
Source:https://en.wikipedia.org/wiki/Tagetes_erecta

Chrysanthemum indicum L.
Source:https://tr.wikipedia.org/wiki/Kasımpatı

Gladiolus grandiflorus L.
Source:https://en.wikipedia.org/wiki/Gladiolus

Lonicera japonica Thunb. It continued to increase the dry biomass and chlorophyll content of leaves and roots at low concentrations of Cd (0-50 mg L-1), but at the highest concentration of Cd, a decrease in dry weights compared to the control was detected. The researchers stated that this herb would be recommended as a Cd-hyperaccumulator due to its high tolerance and capacity to accumulate Cd (Liu et al., 2009).

Cr accumulation in plant organs was mostly in the form of roots> leaves> petioles. Higher Cr accumulation was observed in the absence of Cu. A significant decrease in chlorophyll content was observed in plants exposed to Cr. At the end of the study, the *Nymphaea spontanea L.* plant was determined to accumulate heavy metals (Choo et al., 2006; Lajayer et al., 2019).

Lonicera japonica Thunb.
Source:https://en.wikipedia.org/wiki/Honeysuckle

Nymphaea spontanea L.
Source:https://tr.wikipedia.org/wiki/Nymphaea

Increasing environmental pollution has caused it to be observed in perennial plants. Trees used in parks and gardens It is very important to examine the damage to the plants by the exhaust gases of the trees, especially those caused by vehicles. High concentrations of Fe, Zn, and Cr have been observed in industrial areas. While high concentrations of Pb, Cu, and Ni can be found near highways. Palm leaves have been proposed for biological monitoring of HMs contamination. High levels of Al, Fe, Cu, Zn and Pb have been detected in the leaves of *Quercus ilex* L. due to motor vehicle emissions. Among the species studied, P. nigra has been proposed as a tolerant biomarker plant due to its high Pb and Zn accumulation and low toxicity effect (Baycu et al., 2006; Gratani et al., 2008; Al-Khashman et al., 2011; Lajayer et al., 2019).	 *Populus nigra L.* Source:https://en.wikipedia.org/wiki/Populus  *Phoenix dactylifera L.* Source:https://en.wikipedia.org/wiki/Phoenix_(plant)  *Quercus ilex L.* Source:https://en.wikipedia.org/wiki/Phoenix_(plant)

RESULT AND DISCUSSION

The natural environment, which was considered to be at the beginning of the world's resources and with the development of technology, as unlimited and inexhaustible, has been damaged. This situation puts the use of natural resources at risk. The dispersion of natural resources is important for future life and generations. Also, the destruction and pollution of natural resources do not only affect the efficiency of natural resources but also does not adversely affect living things. To preserve the natural balance and ensure its sustainability, the materials to be used to improve the contaminated areas and the principle of performing the process are not contrary to nature, which increases the acceptability of the methods. Phytoremediation technology focuses on healing with plants, does not disturb the balance of nature, has high feasibility, and aims to cleanse by breaking down, fixing, and removing contaminants in water and soils. For this technique to be successful, the intensity of the pollution, the selection of tolerant and accumulative plants, the preparation and monitoring of the suitable environment for the development of the plant, and the planning of the recovery period are among the issues to be considered.

Protecting the environment from pollutants and improving polluted environments are necessary for the sustainability of the ecosystem. Phytoremediation, which realizes all these phenomena, is a valuable improvement technology to be researched, developed, and transferred to the application. At the same time, using the methods of plant biotechnology, the data obtained as a result of researching candidate genes or proteins for phytoremediation with proteomics and genomic technologies will enable new plants for metal metabolism in plants and remediation of metal contaminated areas. development is very important. Most studies of soil nutrient cycling have focused on essential nutrients in economically important plant species, but our knowledge of the cellular mechanisms of uptake and accumulation of heavy metals in ornamental plants is relatively limited and requires further investigation.

Besides the ability to remove heavy metals from contaminated areas, it would be better to choose ornamental plants either from the same geographic location or regions with similar climatic parameters that could potentially have local adaptations. We should also keep in mind that a reduction in plant growth and biomass may be inevitable in some ornamental plants as well, due to the high toxicity of heavy metals, particularly heavy metals (Hg, As, Pb and Cd) that are not required for plants. However, this potential reduction in growth can be compromised to a certain extent, since heavy metals can cause diseases if they reach plants and animals in the food chain. For this reason, using ornamental plants as a savior in such polluted soil and water resources will seriously secure future generations.

In the light of this information, it should be aimed to increase the future studies on the use and development of plant species and varieties resistant to heavy metals to improve and ensure the sustainability of heavy metals, contaminated soil, and water resources.

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CHAPTER 12

ORNAMENTAL PLANTS AND THEIR HEAVY METAL ACCUMULATION

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INTRODUCTION

Parallel with globalism, the ornamental plant industry is growing rapidly. On a global scale, the production of cut flowers and potted plants is carried out on a total area of 650,000 ha and approximately 75% of this production area is in the Asia/Pacific region. All of America, Africa, Europe, and the Middle East constitute the remaining 25% of production area. Middle East has the least amount of production in terms of hectares (Anonymous, 2020).

Ornamental plants directly affect the psychological and mental state of people; in addition, they are also used for afforestation and decoration of urban areas. The cultivation of ornamental plants is an industry of billions of US$ globally and is a major market as a source for tourism (Tao et al., 2015). In the competition between world markets, efforts have been directed at expanding production areas and increasing yields. However, unfavorable conditions, such as the decrease in available agricultural areas, serious levels of pollution, and adverse conditions generated from global warming, do limit the production of ornamental plants.

Ornamental plants can be grown in various shapes, sizes and colors for decorative purposes, taking into account the different climatic features suitable for the desired garden structure (leafy, flowering, fragrant). A wide variety of plant species, from herbal and woody to low and tall, aquatic or terrestrial, are included among ornamental plants (Liu et al., 2018). Besides the purpose of decorating the environment, they also take important roles in social, cultural, and human aspects, environmental health, and tourism (Özay & Mammadov, 2013). Especially in recent years, new agricultural production models that are based on seeing problematic areas as productive alternative resources have been developed, with the view that ornamental plants may very well have a major role in these models (Wu et al., 2011; Nakbanpote et al., 2016). For this reason, the root, stem, and leaf morphology of ornamental plants should be evaluated, and their place in the planning of production should be determined.

Plants can uptake different elements in soils at varying levels depending on their elemental properties, the soil properties (pH, organic matter, cation exchange capacity, elementary content), and the plant characteristics (age, type, species). To grow and complete their life cycles, plants not only need macro plant nutrients (N, P, K, Ca, and Mg,) but also micro plant nutrients (Fe, Zn, Mn, Cu, and Mo). Interestingly, some plants, including some ornamental plant species, have the unique ability to uptake and accumulate heavy metals, meaning that as metal accumulators they create clean healthy soils (Sun et al., 2011; Gülser et al., 2011; Wang et al., 2012; Cui et al., 2013; Liu et al., 2018).

This current paper reviews the studies related to ornamental species having heavy metal accumulating abilities, their growth in polluted land and conversion of these areas to productive fields. It is believed that growing ornamental species in contaminated soils will generate both economic and ecological benefits.

1. PHYTOREMEDIATION AND ORNAMENTAL PLANTS USED IN PHYTOREMEDIATION

A major part of environmental problems has arisen from the mismanagement of nature. Pollutants in the atmosphere can move and deposit at very long distances from their source via air movements and other atmospheric events, polluting vast areas of aquatic and terrestrial environments, and consequently contaminating a wide range of herbal, livestock, and fishery products. Pollutants from industrial wastes (cyanide, copper, mercury, lead, cadmium, and arsenic), from agricultural pollutants (fertilizer wastes, pesticides) and from house hold wastes (detergents) are resistant to natural breakdown and can accumulate in soils. In addition, the soils could be contaminated when heavy metals in the rocks naturally weather and pass to water or soil environment (Baysal, 1989). However, it is important to remember that soil is one of the most critical natural resources. Yet, it loses its fertility as a result of non-agricultural use, contamination by heavy metals/organic pollutants, and erosion. Soil contamination negatively affects all living organisms, including humans, the last link in the food chain. Today, soil contamination by heavy metals affects approximately 235 million hectares of arable land around the world, and as a result, it is considered a global environmental problem that demands solutions (Bermudez et al., 2012).

It is known that in regions of mining activities or high-industrial pollution factories, there are considerably high concentrations of heavy metals in the roots, leaves, fruits, branches, barks, and wood/trunks of the plants (Shahid et al., 2013; Xiong et al., 2014). Knowing that the entry of heavy metals into the food chain is detrimental to human health, the number of studies on the determination of heavy metal accumulation in plants has steadily increased. This is especially dangerous where residential areas expand to agricultural lands and in some cases completely surround them. In this regard, some people grow plants in their gardens, balconies and pots. Many examples of can be seen in such places; therefore, it is important to restore or stabilize contaminated areas.

The use of plants to clean/remediate heavy metal-containing soils is known as *phytoremediation*, which is a safe alternative to the conventional physical and chemical methods for remediating polluted soils, as these are expensive and may be environmentally hazardous. According to its basic processes and applicability, phytoremediation can be divided into phytoextraction, or phytoaccumulation; rhizofiltration; phytostabilization, or phytoimmobilization; phytovolatilization; and phytodegradation. Phytoextraction or phytoaccumulation is the removal of pollutants from the water or soil by plant roots and their transfer to the above-ground parts. Phytofiltration is the removal of pollutants from surface water or wastewater by plants. Phytostabilization or phytoimmobilization is the immobilization of pollutants in the soil through processes such as uptake, precipitation, and complexation to reduce the amount of toxic metals in the plant root region. In phytovolatilization, plants uptake organic pollutants or certain heavy metals (like Hg, or elements like

Se), convert them into their volatile form and release them into the atmosphere. Phytodegradation is the degradation of organic pollutants without the presence of microorganisms via enzymes released from plant roots, such as dehalogenase or oxygenase (Ali et al., 2013). In phytoremediation, the accumulation capacity of a plant can be determined by calculating its bioconcentration (BCF) and translocation factors (TF). BCF is the ratio of the pollutant concentration in the roots to the pollutant concentration in the soil and the TF is the ratio of pollutant concentration in the leaves to that of the root (McFarlane et al., 2007). Using these two factors, the phytoremediation ability of plants and whether they are suitable for certain phytoremediation applications can be determined. In order for a plant species to be used for phytoextraction, its BCF should be higher than 100 and TF than 1 (Yoon et al., 2006; Liu et al., 2018). Heavy metal tolerant plant species with high BCF and low TF can be used for phytostabilization/ phytoimmobilization of heavy metal polluted areas. Plants with phytostabilization properties uptake metals in their roots and precipitate and immobilize them in different forms, restricting their movement in the soil in this way (Liu et al., 2018). Some of the ornamental plant species that studies have reported to be used for phytostabilization purposes are listed below in Table 1.

Today, the use of ornamental plants has purposes beyond the design of landscape; they are being used to remediate the contaminated soils. One unique advantage of these plants: they do not take place in the food chain when they are used for phytoremediation purposes.

Table 1: Ornamental species used for phytostabilization purposes

Name of ornamental plant	Metal	References
Anthemis stiparum	Cd	Galfati et al., 2011
Calendula suffruticosa	Cu and Zn	Galfati et al., 2011
Calendula officinalis	Cd	Liu et al., 2008
Oenothera glazioviana	Cu	Guo et al., 2014
Chlorophytum comosum	Cd	Wang et al., 2012
Chlorophytum comosum	Pb	Wang et al., 2011
Celosia cristata pyramidalis	Pb	Cui et al., 2013
Osmanthus fragrans	Cd	Zeng et al., 2018
Perennial peanuts	Cu	Andreazza et al., 2011
Tagetes Patula	Cd	Dağhan, 2016

Moreover, ornamental plants are also preferred to be used to enhance the environmental beauty and aesthetics. In this regard, create employment opportunities in these areas. Due to the increase in the use of ornamental plants for phytoremediation in recent years, new plant species with excellent characteristics, such as advanced anatomical features, stress tolerance, and disease resistance, have been produced (Noman et al., 2017). In the many studies conducted on their use in phytoremediation, it has been shown that transgenic ornamental plants are especially resistant to abiotic stress (drought, heat) and biotic stress (pathogens) (Azadi et al., 2016). These studies have also indicated that in places polluted by single or multiple contaminants, the use of ornamental plants for phytoremediation has been quite impressive. Their phytoremediation abilities involve various detoxification mechanisms, such as enzymatic or non-enzymatic (secondary-metabolites) responses, antioxidative responses, the distribution and accumulation of heavy metals in cell-walls, vacuoles, and metabolically inactive tissues, and the chelation of heavy metals with phytochelatins (Nanda & Pradhan, 2019).

The abilities of plants to remove organic or inorganic pollutants and to accumulate, store, or degrade them in natural ways are considered to be environmentally friendly. Plants that can uptake high levels of heavy metals are also known to have defensive reactions and detoxification mechanisms (Nakbanpote et al., 2016). A plant's ability to accumulate heavy metals and distribute them to different parts of the plant depends on plant species, the chemical and biological activity around root environment, the oxidative-reductive potential of plants, pH value, cation exchange capacity, dissolved oxygen, and temperature, and the excretive ability of plant roots (Özay & Mammadov, 2013). Hyperaccumulator plants have transporter proteins in their cell membranes which actively transport heavy metals to different plant parts (Sharma & Dubey, 2005; Özay & Mammadov, 2013).

2. ORNAMENTAL REMEDIATION PLANTS AND HEAVY METAL TOLERANCE

Due to wide variety of ornamental plants, it is very difficult to evaluate them individually. Some are herbal and woody, some are used as a ground cover, some are climbers, aquatic and terrestrial (Figure 1) (Salamon et al., 2006; Cui et al., 2013; Okut, 2019; Capuana, 2020).

The heavy metal accumulation of plants varies depending on the type of metal in the soil, its concentration/total mass, availability/ extractability by the root, the ratio of other elements in the same region, the growth stage of the plant, the climate, and geological and environmental factors (Nakbanpote et al., 2016).

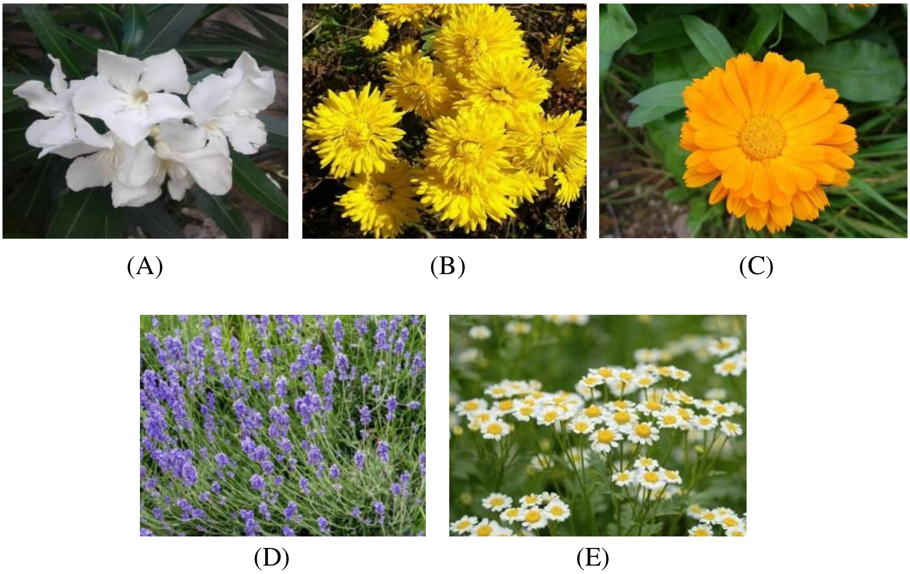

Figure 1: Ornamental species studied for phytoremediation purposes (A: *Nerium oleander*, B: *Chrysanthemum indicum*, C: *Calendula officinalis*, D: *Lavandula angustifolia*, E: *Matricaria* ssp. (Original by Çolak Esetlili)

Preliminary evaluations focusing on the roots, stems, and morphological characteristics of the leaves of ornamental plants to determine their suitability for phytoremediation are ongoing. These evaluations are specifically examining if primarily the plant has a developed root structure, as the uptake of heavy metals or their degradations in the roots are positively correlated with root length, root density, and surface area. Moreover, the root exudates impact the amount and type of microorganisms in the rhizosphere (Cheng et al., 2017). Another remarkable feature of heavy metal accumulations in ornamental plants is the plant height and diameter (Cay, 2015; Liu et al., 2018). The leaves, on the other hand, have significant roles in discharging or evaporating of toxic chemicals. At the same time, the leaf area index plays an important role in biomass increase.

The metal accumulation of ornamental plants also varies according to the different growth stages. *Helianthus annuus* (sunflower) is less vulnerable to heavy metal toxicity in its mature stage than when in seedling stage. Moreover, the growth stage may also affect the accumulation of heavy metals in different plant parts with different ratios. For instance, the order of Cd accumulation in sunflowers in the seedling stage is root> leaves > stalk, while in the mature stage it is stalk > leaves > root > flower (Nehnevajova et al., 2012). In the case of “hyperaccumulator” plants, they accumulate and detoxify very high concentrations of metal ions in their stems (Terzi & Yıldız, 2011). However, metal accumulation varies in different organs of ornamental plants belonging to different species. For instance, when the *Mirabilis jalapa* species was used for Cd phytoremediation in soil, the Cd accumulation in the above-ground parts of the plant was found to be higher compared to the *Iris sibirica* species, which had the highest Cd accumulation in the root (Wang & Liu, 2014; Wang et al., 2018). When metal accumulation is examined at the cellular level, vacuoles are accepted as the main storage site for heavy metal ions. In this regard, phytochelatin-metal complexes are pumped into vacuoles (Salt et al., 1995). The compartmentalization of heavy metal ions in vacuoles forms part of the tolerance mechanism in some hyperaccumulator species (Tong et al., 2004; Lajayer et al., 2019). In some ornamental plants, the accumulation of metals in cell walls, vacuoles, and metabolically inactive (or less active) tissues is an important detoxification mechanism that has the effect of reducing the heavy metal concentration in active cells (Liu et al., 2018). *Alyssum serpyllifolium*, a Ni hyperaccumulator plant, stores 72% of the cellular Ni accumulates in its vacuoles. *Sedum alfredii* Hance, a Zn/Cd hyperaccumulator, Pb accumulator species, uses vacuoles as well as cell walls to detoxify metals (Yang et al., 2002, 2004; Xiong et al., 2004a,b).

In areas with high metal content, vegetation studies should be carried out to limit the movement of heavy metals in the soil and to reduce the dispersion of particles by water and wind erosion. The development of vegetation cover in metal-polluted soils immobilize the metals in the rhizosphere region. The efficiency and success of the phytoremediation process depends on the plant-metal tolerance relationship. Hence, in areas where soil pollution is intense, replanting operations should involve metal tolerant plant species. Table 2 lists some ornamental species, the metal they accumulate, and the part of the plant they store this metal, along with a reference to the corresponding study.

Ornamental plants have the strong ability to extract sunlight, water, and nutrients from the soil. This ability facilitates the growth of these plants, and their metal accumulation. For this reason, the phytoremediation potential of plants should be developed by good agricultural practices, especially by a friendly plant nutrition as this is crucial for plant development and yield. With repeated harvests, the phytoextraction potential of these plants can improve (Nakbanpote et al., 2016).

Jha & Kumar (2017) reported that *Cymbopogon flexuosus* can accumulate hexavalent chromium (Cr VI) and trivalent arsenic (As III) in their body. This species has strong tolerance to heavy metals and the proline content in their leaves increases under stress (Handique & Handique, 2009).

Table 2: Some ornamental plants, the metal they accumulate, and the part they store the metal

Ornamental plants	Metal	Tissue	Reference
Cymbopogon flexuosus	C (VI), As (III)	Roots	Jha & Kumar, 2017
Lolium perenne	Cd	Roots	Liu et al., 2013 Bidar et al., 2007
Trifolium repens	Cd	Roots	Liu et al., 2013 Bidar et al., 2007
Mirabilis jalapa	Cd	Shoots	Wang & Liu, 2014 Begum et al., 2015 Wei et al., 2018
Pyracantha coccinea Roem.	Ni	Branches and leaves	Akguc et al., 2010
Calendula officinalis	Cu	Shoots and roots	Afrousheh et al., 2015
Tagetes erecta	Cd Pb	Shoots Roots	Minisha et al., 2021 Shah et al., 2017
Chlorophytum comosum	Cd	Roots	Wang et al., 2012
Lonicera japonica Thunb	Cd	Roots	Liu et al., 2009
Bidens pilosa	Cd	Shoots	Sun et al., 2009
Pelargonium hortorum	Multi elements	Shoots	Orrono & Lavado, 2009 Orrono et al., 2012
Althaea rosea	Cd	Shoots and roots	Liu et al., 2009
Pteris vittata.	As	Fronds (above ground biomass)	Cao et al., 2004
Tagetes patula	Cd	Shoots	Dağhan, 2016
Pteris quadriaurita	As	Roots	Srivastava, et al., 2006
Schima superba	Mn	Leaves and stems	Yang et al., 2008
Lavandula vera	Cd and Pb	Roots	Angelova et al., 2015

Other studies with the same plant showed that it can also phytostabilize heavy metal polluted soils (Handique & Handique, 2009; Lee et al., 2014; Gautam et al., 2017). Many studies have reported that the *Trifolium repens* and *Lolium perenne* species have the ability to accumulate heavy metals (Bonnet et al., 2000; Caggiano et al., 2005; Bidar et al., 2007). The latter has high dry mass yield and high metal accumulation abilities (Pichtel & Salt, 1998). Arienzo et al. (2004) also reported that this plant can be successfully grown on metal-polluted soils. In *Trifolium repens* and *Lolium perenne*, the concentration of Cd, Pb, and Zn were reported to be higher in roots and stems, and in the latter species, the root's Pb concentration was much higher than that of *Trifolium repens* (Bidar et al., 2007). Another ornamental plant used for phytoremediation is *Mirabilis jalapa*. It is used in Cd polluted soils and accumulates Cd mostly on its above-ground parts (Wang & Liu, 2014). *Pyracantha coccinea* has biomonitoring properties, meaning that it can detect changes in the concentration of Pb, Cd, Cu, Zn, Fe, Mn, and Ni pollutants in its parts. Since its leaves and branches accumulate Cu and Ni, it could be useful for phytoremediation (Akguc, 2010).

Tagetes patula (marigold) is an aesthetic, annual plant that can grow in different soils and climates and easily adapt to changing environmental conditions (Liu et al., 2011; Priyanka et al., 2013). Considering that it is an annual plant and not edible, it is recommended to be grown in areas with high Cd pollution for phytoremediation purposes (Liu et al., 2011; Dağhan, 2016). Moreover, this plant is also beneficial for overall agriculture, as it has a pungent odor that repels harmful pests. The marigold also secretes a nematicide exudate (alpha-terthienyl) from root tissue for the control of root-knot nematodes and lesion nematodes. Due to its allelopathic effect, it can be used as an auxiliary crop or as cover-crop (Prasad & Nakbanpote, 2015).

Studies in recent years have shown that *Calendula officinalis* can accumulate environmentally polluting metals, such as Cu, Cd, and Pb. It was further found that the dry mass and height of *Calendula officinalis* changed according to changes in the Cd concentration in the soil. It showed no toxicity response in a contaminated soil with a 200 mg/kg of Cu content. Its shoot had 349 mg/kg Cu concentration, while its roots had even higher concentrations (459 mg/kg) (Liu et al., 2018). Most Cd hyperaccumulator plants suffer from slow-growth and limited biomass production. However, *Bidens pilosa* has a potential for the phytoremediation of heavy metal polluted soils. During the flowering and maturation periods, the Cd concentration in the stem, leaves, and shoots was found to be higher than the 100 mg/kg threshold concentration value of a Cd-hyperaccumulator plant; the bioconcentration (BCF) and translocation (TF) values were found to be >1 BCF (Sun et al., 2009).

The *Althaea rosea* plant can be used as an alternative crop for remediating Cd polluted soils, especially in urban areas. Besides its aesthetic value, it has significant potential to rehabilitate Cd polluted areas (Liu et al., 2009). In such areas, it is recommended that a plant management strategy be used that involves short-rotation plants and fast-growing trees, like eucalyptus and willow, to create a green belt around the contaminated area (Pulford & Watson, 2003).

CONCLUSION

In recent years, the use of ornamental plants for the remediation of polluted soils has emerged as an excellent alternative to the use of other plants for the same purpose. The following is a list of the benefits of using ornamental plants for phytoremediation:

1. Ornamental plants can be used for the conversion of polluted soils to fertile areas, which would increase the production capacity of the soil (one of the most important natural resources) and contribute achieving sustainable soil management.
2. Many ornamental plants are not edible. This reduces the risk of heavy metal entry into the food chain.
3. Growing ornamental plants in polluted areas would open up an alternative income for the locals and offer additional ecotourism development opportunities.
4. The management and operation of other remediation technologies can be quite complex and difficult. The use of ornamental plants for remediation would further enhance phytoremediation's effectiveness with a new dimension.
5. In addition to environmental clean-up, ornamental plants would also design and refine the landscape.

Although many ornamental plants have high tolerances to a variety of organic and heavy metal pollutants on account of their considerable accumulation capacities, it is important to make a careful selection of pollutant/plant genotype pairs that match to specific environments to ensure a successful strategy of using ornamental plants for remediation. In the future, new studies are expected on the determination of the molecular mechanisms behind the ornamental plants' heavy metal accumulation and transfer in its body, the results of which will serve to further promote the ever-promising potential of these plants on remediation.

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CHAPTER 13

THE IMPORTANCE OF ORNAMENTAL PLANTS IN EROSION CONTROL

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INTRODUCTION

Current developments in the world and the damage caused by humanity to the environment cause the global ecological balance to deteriorate (Akça & Yazici, 2017; Akça et al., 2019a; Yazici & Gülgün, 2020; Temizel et al., 2019). The soil which is the essential resource for the continuation of all living things and the ecosystem must be protected (Temizel et al., 2018; Yaman & Temizel, 2019). Every year in the world and in our country, soil loss and early filling of dams are caused by thousands of tons of fertile soil which was carried to rivers as a result of erosion (Yazici et al., 2013; Gülgün et al., 2015a,b). In Turkey, like the rest of the world, the most important reason for the loss of soil is erosion. The topographical structure, geographical location and climatic conditions of our country have a triggering effect on erosion. Erosion has many negative effects not only on the physical environment but also on the economic conditions and social relations. The purpose of this article is to determine the erosion potential with scientific data for our country and proposing sustainable solutions for local practitioners (Çalışkan & Boydak, 2017; Akıncı Cındık, 2018).

1. EROSION: DESCRIPTION & TYPES

In recent years, a significant decrease in ecological, biological and economic productivity occurs with the increasing effects of erosion, desertification, drought and land degradation as a result of anthropogenic pressures and climate changes. Due to the destruction of vegetation, erosion of soil, water and wind is at crucial levels both in the world and in our country. According to the Worldwatch Institute, in the world, it is claimed that 24 billion tons of topsoil are lost every year. According to ÇEM (GDCDE: General Directorate of Combating Desertification and Erosion) data, 248.6 million tons of soil is transported from agricultural lands due to water erosion in our country. In the study, the solution methods applied in our country to prevent soil loss due to erosion and the plant species used in these methods are mentioned. As a result, suggestions on the subject are stated (Dunning, 1995; Ersan et al., 2017).

There are various national and international studies on the definition of erosion. Erosion is the process of transportation and accumulation of the existing material to another region by various internal or external effects. It is the process that the materials on the soil and the earth are eroded from the place where they were formed by various erosional factors as a result of the separation of rocks and plants from the earth's crust by physical (mechanical), chemical and biological factors under natural conditions. In our country, it is possible to see examples of water and wind erosion in every intensity (Görcelioğlu, 1996).

1.1. Water Erosion

In our country, it is the most common and effective erosion type. Especially in sloping areas, where the vegetation is weak or disappeared, the rain breaks off the soil and creates floods that flow in the form of floods with rainwater and can cause great material damage (Figure 1,2). In addition, the topsoil which is rich in nutrients reduces soil fertility as a result of water erosion, and it is not possible to regain the same fertility rate in a short period of time (Acar et al., 2006).

Figure 1: Examples of lands where water erosion occurs (URL-1; URL-2)

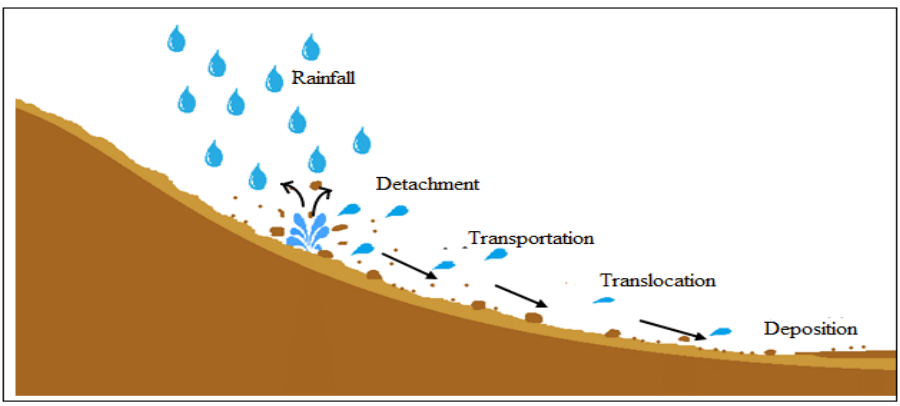


Figure 2: How water erosion occurs (URL-3)

According to the data obtained from the Dynamic Erosion Model and Monitoring System of the Ministry of Agriculture and Rural Affairs; water erosion is severe in 6.7% of Turkey's surface area; severe at 5.97%; medium at 7.93%. It shows a light distribution of 19.13% and a very light distribution of 60.28% (Figure 3). In addition, the elements that affect water erosion in Turkey are 47.55% topography, 34.82% vegetation, 14.26% rainfall, 3.36% soil effect (Figure 4).

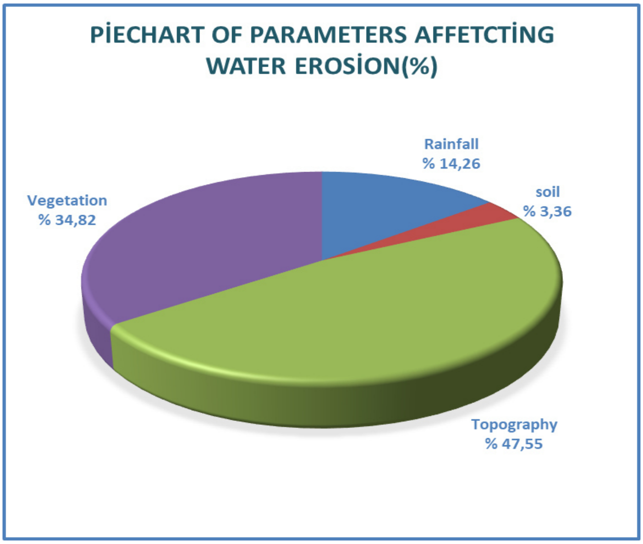


Figure 3: The elements that affect water erosion in Turkey (Anonymous, 2018)

One of the most important causes of erosion is the removal of natural vegetation. In addition; this complication arises from the uneven terrain and very steep slopes, incorrect land use, plowing the soil in the same direction with the slope, converting forest areas into agricultural land, destroying natural vegetation, fires, overgrazing, the effect of the climate, excessive rainfall, the impact of the bedrock and non-resistant layers plays.

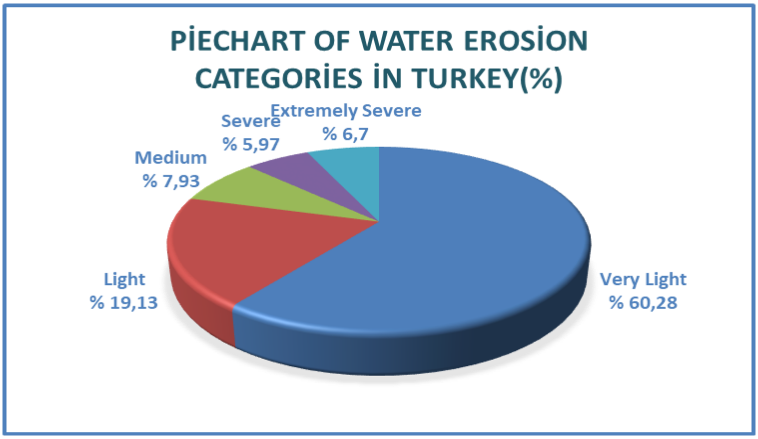


Figure 4: Water erosion categories in Turkey (Anonymous, 2018)

As a result of these factors, the natural balance deteriorates, the yield value of the land decreases, dams fill up, and agricultural areas are filled with sand and gravel. When evaluated in terms of land use, 4.17% of the displaced land in our country belongs to forests, 38.71% to agriculture and 53.66% to pasture areas (Figure 5).

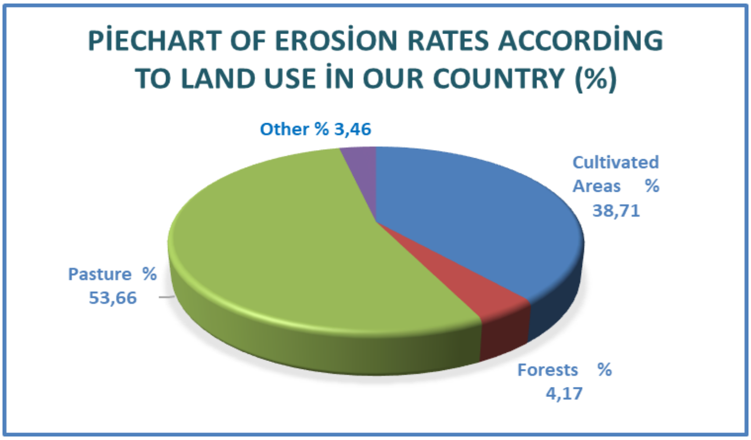


Figure 5: Erosion rates according to land use in Turkey (Anonymous, 2018)

Figure 6 demonstrates the soil erosion rates in the world. The map shows that the regions with the highest risks are particularly India, China, sub-Saharan Africa and South America, where deforestation is intense.

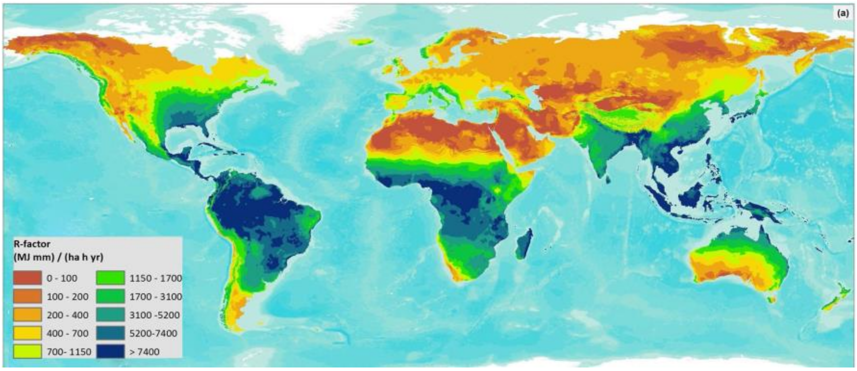


Figure 6: The map shows that the regions with highest risks (URL-4)

1.2. Wind Erosion

Wind erosion is the most common type of erosion after water erosion. Especially in regions with arid and semi-arid climates (annual precipitation is below 400 mm); It is effective in South East Anatolia, Central Anatolia and Thracian Regions. Due to the loose soil and high amount of sand and silt in these regions; it occurs as a result of the transportation and accumulation of soil in flat and wide areas with the effect of strong wind. If there are no factors such as vegetation and barrier to slow the wind speed in flat and near-flat open lands, wind erosion will be even more effective.

According to SYM (DEM: Digital Elevation Model) prepared by the Ministry of Agriculture and Forestry, flat or near flat areas with a 6%

slope (or less) were determined in Turkey and a map was created based on the amount of sediment transported via wind (İnce et al., 2018) (Figure 7).

It indicates that Central Anatolia, Southeastern Anatolia and Thrace regions have a high rate of sediment transportation. Figure 8 shows the images of wind erosion; (a) Agricultural land (URL-5), (b) Natural area (URL-6).

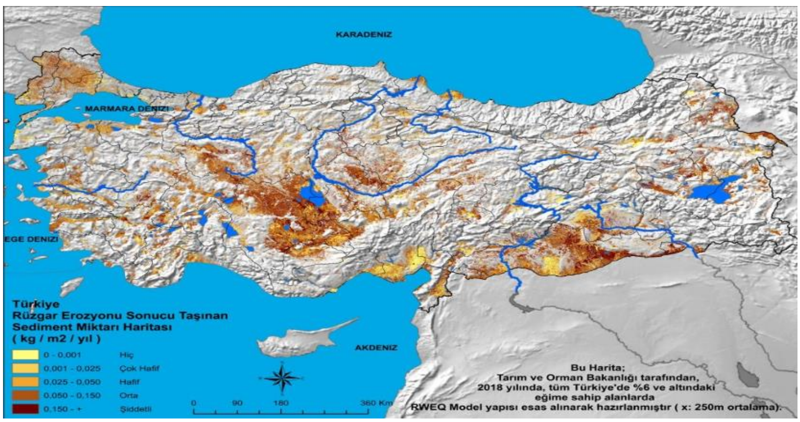


Figure 7: Map based on the amount of sediment transported via wind (Anonymous, 2018)

(a) (b)

Figure 8: (a) Agricultural land; (b) Natural area

1.3. Erosion Caused by Snow Avalanches

An avalanche is an event that occurs as a result of the loading of snow masses with freshly falling snow in mountainous areas covered with snow (Figure 9). An avalanche erosion is the snow flow that transports and deposits soil, rock debris and trees. In our country, it is mostly seen in the North-Northeast and Eastern Anatolia Regions. It is very rare that large and destructive avalanches occur on slopes covered with dense forests (Kurt, 2017; Görcelioglu, 2003; Başyiğit & Dinç, 2016). When it comes to avalanche formation, the riskiest percentage of the slope is between 35-40% (Naylor et al., 2002; Rudolf-Miklau et al., 2015).

Figure 9: Case of an erosion caused by snow avalanches (URL-7)

2. EROSION PREVENTION METHODS AND THE PLANT SPECIES TO BE USED

The purpose of all control methods in erosion control is to increase soil fertility, create urban or extra-urban green areas, meet the recreational needs of the public, protect biological diversity and water resources;

reduce and noise pollution; extend the life of dams. Although the factors causing erosion differ; in the "water" and "wind" erosion areas that are active, the main reason is the steep slope, insufficient vegetation and soil characteristics. The first step of combating surface erosion is landscape terracing. In order to prevent the erosion of rainwater on sloping lands, the gradings formed parallel to the curves are called “terraces”. In sloping lands, the structures created by digging the slope along contours and piling the extracted soil into the lower part are called “terraces”. This process is called "terracing". It is one of the oldest methods used for obtaining agricultural land in areas where the protection of biodiversity is crucial, the population density is high and the small-sized cultivated areas. Terracing works are done in areas where the slope of the land is high. In general, if the slope of the land is above 5%, it is accepted that the erosion risk arises. There is no need for terracing of the land in regions with a slope of 5-12, erosion prevention can be achieved by vertical tilling. Landscape terracing can be only implemented if the slope range is between 15% and 60%. Terraces, which are one of the most important points of combating erosion; vary according to the slope of the land, the contour of the area and the soil characteristics (Çalışkan & Boydak, 2017; Kaba, 2019).

2.1. Sloped Terracing

Depending on the plant growing conditions of the region, terraces vary according to their construction and purpose. Terraces created in order to reduce the surface water flow, especially in regions with high rainfall, are called "sloping terraces" (Figure 10). Purpose in sloping terraces; It is to keep the rainfall water on the slopes at short distances, to flow out of the slope and to prevent soil transport.

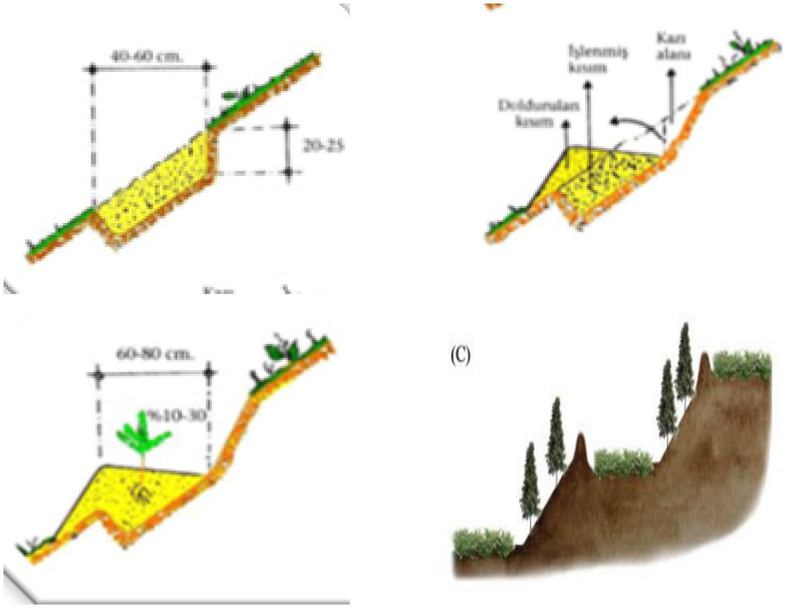


Figure 10: Planting practices in sloped terraces (URL-8)

Thus, creating a suitable environment for afforestation. Sloping (diversion) terraces collect the runoff water before it gains the power to wash away, disallow the lined-erosion by stopping the surface erosion and prevents them from being scoured (Görcelioğlu, 1996). It is a more reliable facility against floods and overflows due to the larger size of the pouring terraces and the ability to drain the water that is held. Also, it is preferred in arid and semi-arid regions due to its contribution to the water presence in the soil. Sloped terraces take place in the erosion zones where afforestation cannot be made in erosion zones to protect the flat terraces (Figure 11).

Figure 11: Afforestation in sloped terraces (URL-9,10,11)

In addititon, sloped terraces block uncontrolled water flow from rocky or agricultural land, pasture, road, etc. When creating the terrace; the soil is tilled (depth: 35-40 cm, width: 60-80 cm) in the areas with a slope of 12-60%. The planting of proper species must be executed in parallel with the formation of slope curves considering intervals of the species to be planted.

2.2. Flat Terraces

Flat terraces are applied in the regions with hot and dry climates; their aim is to prevent surface runoff and protect the soil and retain water. Slope-free terraces; They are terraces with zero slope according to contour lines. It is more preferred in soil sensitive to erosion and conditions with high infiltration ability. Flat terraces are generally divided into three (Luuk & Freddy, 2018).

Gradoni-Type Terraces: It is applied in areas with a slope of up to 60%. The width is between 60-80 cm and a slope of 20-40% is given towards the terrace surface (Figure 12).

***Cut-off terraces*:** It is a type of terrace applied in rainy areas with a slope of less than 40%. It is built to be 30 cm in width and 20 cm in depth (Figure 13).

Gradoni-Type With Ditches: It is applied in areas with a slope of up to 60%. The width is between 15-20 cm, the depth is 30-35cm (Figure 14).

Bench Terrace; These applications can be done by machine in areas with a slope of up to 70% (Figure 15). It is used in terracing to stabilize the lower parts of the soil with an average width of 4 m. It consists of two types of material, stone and soil. In these terraces, leafy plant species are preferred according to the fill slope, and coniferous plant species are preferred in the middle of the terrace (Morgan, 1986; Yeşilkaya & Cengiz, 1996).

Terracing practices can be in different forms. The nature of the soil in the erosion areas, the slope, the way of use, the slope of the land, the hydraulics of the region and the application possibilities in the environment are important factors that determine the type of the terrace (Figure 16).

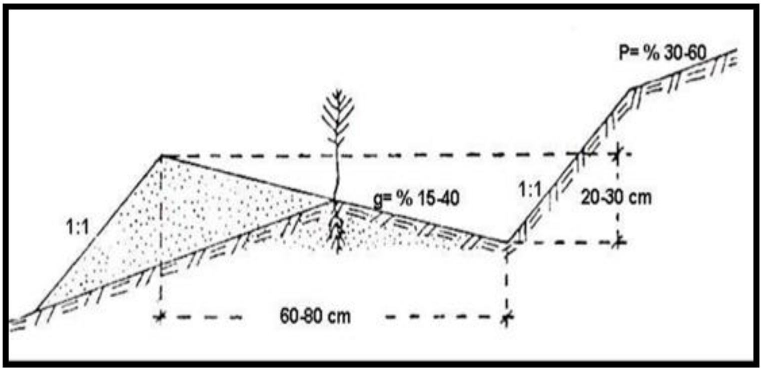


Figure 12: Gradoni-type terraces (URL-12)

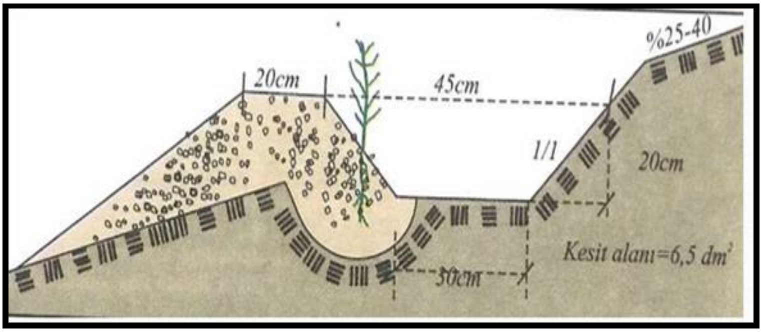


Figure 13: Cut-off terraces (URL-13)

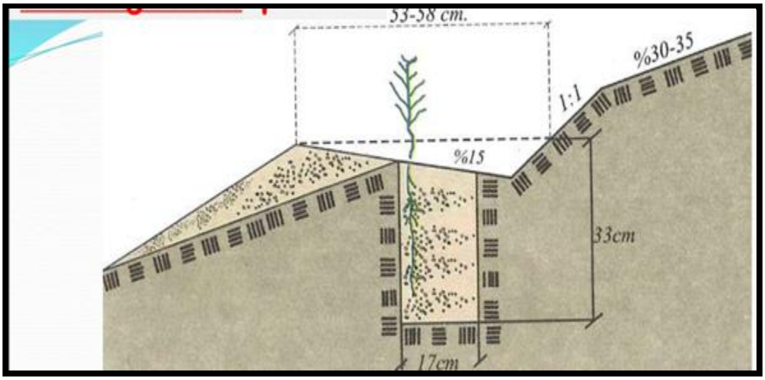


Figure 14: Gradoni-type with ditches (URL-14)

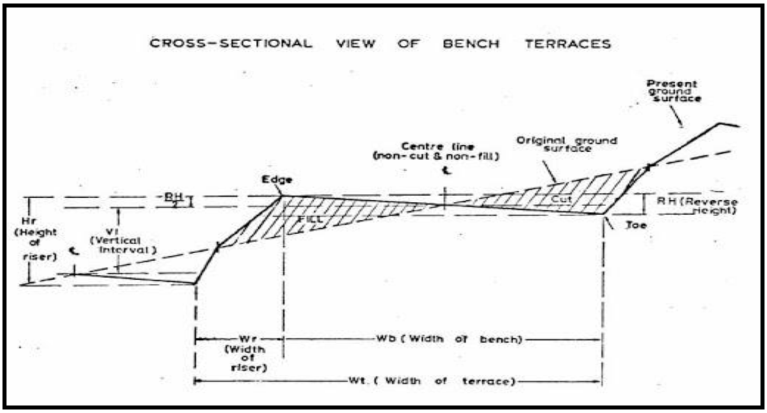


Figure 15: Bench terrace type (URL-15)

Soils with fertile characteristics are lost as a result of wind erosion. As a result, the evaporation accelerates with the effect of wind and soil moisture decreases. Therefore, decreasing soil moisture slows down plant growth. In combating wind erosion; protective forest belts and windscreens, windbreaks and dune stabilization are the key points.

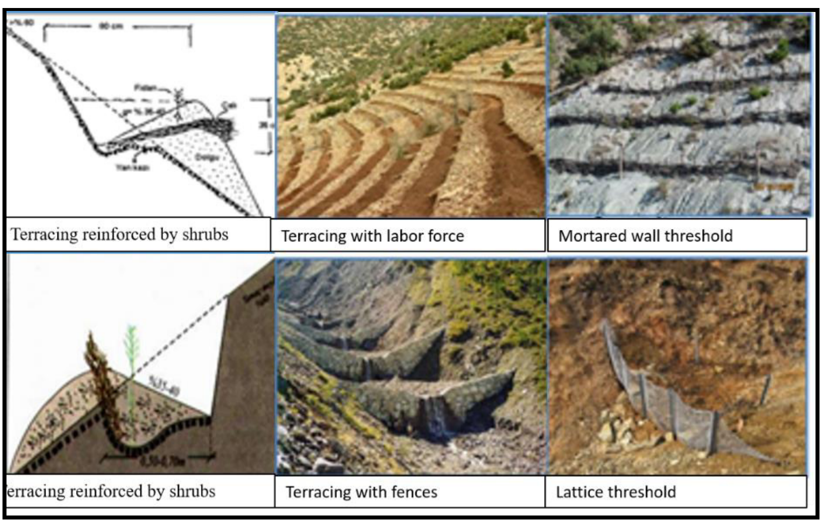


Figure 16: Terracing examples: a) terracing reinforced by shrubs (b) terracing with labor force (c) terracing with fences (d) mortared wall threshold (e) lattice threshold (URL-16,17,18)

2.3. Protective forest belts, windscreens and plant species used in the study

Protective forest belts are 30-60 cm wide and consist of 20-50 tree rows. Wind curtains are 5-20 m wide and consist of 3-10 tree rows on average (Figure 17). Wind curtains protect the upper and lower layers of the soil. It also protects wildlife, agricultural products, animals and people from the negative effects of the wind. Natural plant species should be preferred in wind screening. However, the features mentioned above should be taken into consideration. Fringe root-developing species such as *Eucalyptus, Ailanthus, Acacia* and *Populus* should not be included in the edges of the ribbon. It is best if the strips are in the form of a leafy + coniferous mixture. Among the leafy species that can be applied in wind screening in our country, there are *Populus nigra, Eucalyptus globulus, Salix alba, Fraxinus excelsior, Alnus glutinosa, Quercus* spp.; conifers include *Cupressus sempervirens, Pinus brutia, Thuja occidentalis* (Yavuzsefik & Uzun, 2005).

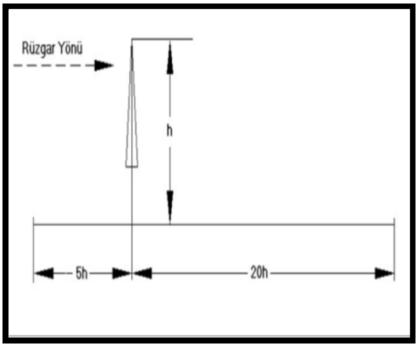


Figure 17: Windscreening in cultivated areas (URL-19,20)

2.4. Windbreaks

Rows of trees or shrubs that reduce wind power are called windbreakers. Windbreaks can reduce erosion, increase crop yields and protect cattle from cold and heat. In front and behind the wind break, the wind speed cutting efficiency is around 20% at a distance of 10-12 times its height (Figure 18).

Figure 18: Windbreaks in dune areas (URL-21)

2.5. Dune Stabilization

The reason dunes are anchored is to destroy the source of sand and keep the dunes in one place (Figure 19). Where strong winds blow from only one direction, rows of fences should be erected perpendicular to these winds. In this way, wind erosion can be stopped up to 20 times the row height. Perennial tree species such as *Acacia, Tamarix, Zizyphus, Parkinsonia, Prosopis, Ampliceps* and *Eucalyptus* have been used to improve vegetation in the moving dune area affected by wind erosion (Sinha et al., 1999; Karaoğlu, 2018).

Figure 19: Dune s tabilization (URL-22)

3. ROLE OF PLANT USE AND PLANT SPECIES TO BE USED IN EROSION PREVENTION METHODS

The most important factor in reducing and preventing the effect of erosion is vegetation. If planting applications are executed according to the region and geographical conditions; it was found that the total soil loss rate in the uncultivated and vegetated areas is between 30.2% and 94.3%; the total surface runoff decreased between 18.4% and 66.4% (Martinez et al., 2006). The effect of vegetation in combating erosion is divided into two as bioprotection and biostructuring (Naylor et al., 2002; Akça et al., 2019b,c,d).

Effects of plant vegetations on bioprotection and biostructure;

- To prevent soil loss by minimizing the effects of raindrops with the help of above-ground portions.
- To prevent water and air movements on the surface by covering the vegetation at a certain height on the soil surface.
- Preventing the movements of soil masses with the help of roots.

- To reduce the evaporation and susceptibility to erosion by creating a shadow effect in the soil.
- To increase the amount of organic matter and water holding capacity of the soil with plant residues.
- Contributing to the ecological cycle through transpiration.

3.1. Plant Species To Be Used In Erosion Prevention Methods

Many plant species can thrive in extreme conditions and resist harsh conditions. These types of plants are prioritized in combating erosion. Some important species of these plants that can grow in our country are given below.

Trees: *Ailanthus altissima, Quercus infectoria, Robinia pseudoacacia, Salix alba, Salix nigra, Salix purpurea, Pinus halepensis, Pinus elderica, Pinus brutia, Quercus* sp., *Acer negundo, Alnus incana, Betula pendula, Pinus nigra, Pinus pinea, Pinus silvestris nigra, Populus tremula, Prunus mahalep, Prunus amygdalus, Ziziphus zizyphus, Robinia pseudoacacia, Eucalyptus camaldulensis* where annual rainfall is 300 mm; In arid regions, tree species such as *Fraxinus excelsior, Gleditsia triacanthos* and *Robinia pseudoacacia* have been suggested (Figure 20). These tree species should be given priority, as they also meet the nitrogen needs of the trees by connecting the nitrogen of the air to the soil with the help of their roots. These plants shown in Table 2.

Small Trees and Shrubs: *Berberis crategina, Maclora pomifera Cistus* spp., *Crataegus* spp., *Euonimus europeus, Ligustrum vulgare, Lonicera xylosteum, Pyracantha coccinea, Rhus typhina, Rosa canina, Tamarix* spp., *Vitex agnuscastus, Prunus dulcisi, Crataegus monogyna, Pistacia vera, Celtis australis, Rosmarinus officinalis, Cotinus coggyria, Rhamnus pallasii, Cotoneaster nummularia, Cerasus mahaleb* var. *mahaleb, Spiraea crenata, Salix caprea*. These plants shown in Table 2.

Herbaceous plants: *Trifolium pratense, Trifolium repens, Lotus corniculatus, Tussilago farfara, Melilotus albus, Melilotus officinalis, Medicago sativa, Astragalus* spp., *Festuca rubra, Festuca ovina, Koeleria cristata, Lolium perenne, Silene* spp., *Dactylis glomerata, Agrostis alba, Cynodon dactylon, Poa pratensis, Poa trivialis, Vicia* spp., *Chrysopogon zizanioides, Thymus vulgaris, Stipa tenacissima, Anthyllis cytisoides.* These plant shown in Table 3 (Köseoğlu & Özkan, 1984).

Table 1: The group of ornamental plants is one of there trees that can be used in erosion control

								
Salix babylonica (URL-23)		*Ailanthus altissima* (URL-24)		*Pinus elderica* (URL-25)		*Gleditsia triacanthos* (URL-26)		*Fraxinus excelsior* (URL-27)
								
Salix alba (URL-28)		*Robinia pseudoacacia* (URL-29)		*Pinus brutia* (URL-30)		*Quercus* sp. (URL-31)		*Betula pendula* (URL-32)

Table 1. The group of ornamental plants is one of there trees that can be used in erosion control (Cont.)

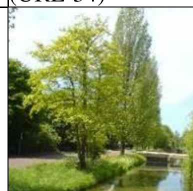
								
Salix nigra (URL-33)		*Acer negundo* (URL-34)		*Pinus halepensis* (URL-35)		*Pinus pinea* (URL-36)		*Prunus mahalep* (URL-37)
								
Salix purpurea (URL-38)		*Alnus incana* (URL-39)		*Pinus nigra* (URL-40)		*Ziziphus zizyphus* (URL-41)		*Prunus amygdalus* (URL-42)

Table 2: The group of ornamental plants is one of there shurbs that can be used in erosion control

								
Berberis crategina (URL-43)		*Maclora pomifera* (URL-44)		*Cistus* spp. (URL-45)		*Crataegus* spp. (URL-46)		*Euonimus europeus* (URL-47)
								
Ligustrum vulgare (URL-48)		*Lonicera xylosteum* (URL-49)		*Pyracantha coccinea* (URL-50)		*Rhus typhina* (URL-51)		*Rosa canina* (URL-52)

Table 2: The group of ornamental plants is one of there shurbs that can be used in erosion control (Cont)

								
Tamarix spp. (URL-53)		*Vitex agnuscastus* (URL-54)		*Crataegus monogyna* (URL-55)		*Rosmarinus officinalis* (URL-56)		*Cotinus coggyria* (URL-57)
								
Piraea crenata (URL-58)		*Rhamnus pallasii* (URL-59)		*Cerasus mahaleb* var. *Mahaleb* (URL-60)		*Celtis australis* (URL-61)		*Pistacia vera* (URL-62)

Table 3: The group of ornamental plants is one of there herbaceous plant that can be used in erosion control

								
Trifolium pratense (URL-63)		*Trifolium repens* (URL-64)		*Lotus corniculatus* (URL-65)		*Tussilago farfara* (URL-66)		*Melilotus albus* (URL-67)
								
Astragalus spp. (URL-68)		*Medicago sativa* (URL-69)		*Silene* spp. (URL-70)		*Dactylis glomerata* (URL-71)		*Agrostis alba* (URL-72)

Table 3: The group of ornamental plants is one of there herbaceous plant that can be used in erosion control (Cont)

								
Cynodon dactylon (URL-73)		*Poa pratensis* (URL-74)		*Vicia* spp. (URL-75)		*Chrysopogon zizanioides* (URL-76)		*Thymus vulgaris* (URL-77)
								
Stipa tenacissima (URL-78)		*Anthyllis cytisoides* (URL-79)		*Festuca ovina* (URL-80)		*Koeleria cristata* (URL-81)		*Melilotus officinalis* (URL-82)

CONCLUSION

Around 25-50 billion tons of soil is transported a year by erosion in the world. In Turkey, the ongoing intensive water erosion affects 78% of our land. As a result, 500 million tons of land are transported to the seas annually. Especially, the cost of erosion in agricultural land to our country reaches 6.7 billion dollars (URL-83).

- According to the studies of Acar et al. (2006), maximum soil protection can be achieved by using pasture plants in erosion control activities. When pasture plants are used together with *Pinus brutia* and *Pinus pinea* species, it is reported that there is no change in topsoil loss.
- It is stated that *Thymus vulgaris* (thyme) reduces soil loss by 97% and surface runoff by 91% (Martinez et al., 2006; Çakar, 2019).
- Runoff parcels have been placed at determined points on the slopes covered with *Olea* sp. (olive) trees on Lesbos Island. It is mentioned that typical maquis vegetation types are quite effective in reducing water erosion (Koulouri & Giourga, 2007).
- According to TUIK data; between the years 2001-2019, it is shown that the pasture areas in our country were 14,617,000 ha in total and did not change at all (URL-84). The yield potential of the rangelands is not sufficient for the increasing number of cattle and sheep. As a result of overgrazing, the vegetation cover of the pasture areas has weakened and they have become areas open to erosion.

- Although erosion cannot be prevented completely, it has been taken into consideration that its impact can be reduced by preventive studies in these areas and the following suggestions have been made;
- In combating erosion, it is important to stop runoff in erosive areas and to establish new vegetation in these areas. The fact that plants are not included enough in the measures taken and that existing engineering methods are extremely expensive are among the troublesome issues.
- Each province should prepare "Model study erosion maps" according to its potential.
- In combating water and wind erosion, quantitative evaluations should be made in line with the regional scale by using Geographical Information System (GIS) and Remote Sensing (RS) methods.
- Lands in each erosion zone should be determined according to their ability classes. Farmers should be informed and trained by analyzing the slope, temperature and precipitation data of the region. In wind erosions; wind curtains to be positioned according to the direction and strength of the prevailing wind should be provided. The pasture areas of the region should be proportional to the number of animals per unit area. Planting studies should be carried out together with landscape architecture and engineering studies.

- Since it is more economical and easier to prevent erosion; precautions should be taken before erosion starts. (Karaoglu, 2018).

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CHAPTER 14

EVALUATION OF SOME WILLOWS (*Salix* L.) SPECIES IN SUSTAINABLE ENVIRONMENT AND LANDSCAPE REPAIR STUDIES

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INTRODUCTION

In the world, intense construction, technological and economic developments, and rapidly depleted resources have caused the deteriorating natural balance in recent years. Therefore, the environment and landscapes that have been destroyed over time require repair works.

There are many benefits, such as increasing the soil fertility of afforestation works, ensuring food security, establishing green areas and city forests around cities, reducing air and noise pollution, preventing dust transport, erosion, preserving water resources, extending the life of dams, meeting the need for wood raw materials of our country, recreation, meeting the recreational needs of the people and protecting biological diversity (Anonymous, 2020). It is known that expanding cities and green areas, improving air quality, reducing noise pollution, preventing uncontrolled urban sprawl and other beneficial effects play an important role in creating quality landscapes. According to Erdoğan Onur & Demiroğlu (2016), in the extension of these green areas, landscape planning, design and management model the nature itself within the framework of the ecological approach, and produces solutions that are compatible with natural processes and the structural and ecological characteristics of the area. Selection of plants that play an active role in creating these areas; the use of species suitable for the local and existing ecological structure and the introduction of species diversity, limited intervention to natural development, protection of natural appearance, preference of species that will create habitats for wildlife, protection of existing habitats should be done within the framework of landscape restoration.

As a result of the change and development in the urban structure resulting from the reflection of ecological principles to the urban green space system, it is very important to rearrange the idle areas in the urban structure with an ecological understanding. Abandoned power stations, old mining areas, abandoned railroad areas, and areas that are filled with water by remaining pits, steep and unsuitable for construction can be examples of urban idle areas. When such areas remain empty for a long time, they are occupied by natural plant and animal species, then a natural habitat is formed in these areas and creates potential for the organization of ecological green areas (Emery, 1986; Erdoğan Onur & Demiroğlu, 2016). An example of evaluating idle areas with a sustainable approach is "Igneborne Hill" in London. The hill, which is an old garbage storage area with 45 ha area, was restored; It has been turned into a focal point for recreational activities and habitat connections have been established with the surrounding valleys (Landscape Institute, 2013).

According to Ruff & Teragay (1982), Emery (1986), in the vegetative design of the area, natural plant species that can develop without intervention, need no renewal, require less maintenance and spraying, and have relatively less water need should be preferred. The use of plants resistant to local climatic conditions is emphasized in all sample areas in the reports published by the Landscape Institute and SSI, two non-governmental organizations that carry out important studies on the subject. It is recommended to use natural ornamental plant species in order to support the natural wildlife in the mentioned sample area (SSI, 2009; Landscape Institute, 2013).

Today, some ornamental plants are used in soil reclamation and erosion prevention activities, targeting different landscape restoration purposes. Naturally occurring erosion can be exacerbated by human interventions, especially in vegetation. For this reason, afforestation and improvement of the existing vegetation are of great importance in planning areas where landscape restoration works are needed such as preventing erosion, soil rehabilitation and reducing the toxic effects of environmental pollutants. With this study, the willow plant, which has an important place in sustainable environment and landscape restoration studies, has been evaluated. Willow is a very valuable plant in terms of natural resources in the world.

In particular, this plant species is a valuable plant that varies according to the conditions and geographical location of each region in terms of number and diversity, many species are found naturally on the waterfront, resistant to extreme climatic conditions, and mostly solitary in landscape design works other than repair.

1. *SALIX* L. AND THEIR DISTRIBUTION

It is known that the Salicaceae family has three genera such as *Salix* L., *Populus* L. and *Chosenia* L. (Yaltırık & Efe, 1994; Yıldız &Aktoklu, 2010). Among these, *Salix* L. has the most species diversity is genus. *Salix* L. genus, which has around 400 taxa, shows natural distribution in large communities, mostly in the Northern Hemisphere and slightly in the Southern Hemisphere (Rushforth, 2002). 27 species of willow can grow naturally in the Northern Hemisphere's temperate climate and some types of cold is located in Turkey (Çağlar, 2003; Bıçakcı et al., 2014). According to Esen (2010), this plant reveals that in the Iran-Turan phytogeographical region, *Salix aegyptiaca* species, 1600-2200 m, along the Posof Stream and between Asmakonak-Çamyazı Village, is among the hygrophile vegetation elements that spread along the river side, stream slope and lake side. The most important willow species used as naturally occurring and ornamental plants in Turkey *Salix alba, Salix viminalis, Salix fragilis, Salix cinerea, Salix purpurea, Salix amygdaline, Salix caprea, Salix aegyptiaca* and *Salix incana* species were determined (Kayacık, 1963). Willows have a high demand for light and are sensitive to acidic soils. There are also species that grow in arid soils. In addition, they are resistant to frost (Gökmen, 1970). Willows have a high demand for light and are sensitive to acidic soils. There are also species that grow in arid soils. They are also resistant to frost (Gökmen, 1970). Some tropical species are found in the mountainous parts of the region, close to the forest border. They can be tree and shrub shaped, sometimes smaller (such as *Salix reticulata* L. in Alpine regions). Since it is difficult to produce from seed (Akman et al., 2007), it is easier to grow many of the species with the help of cuttings or root shoots. The vegetative breeding of the species mentioned above provides great convenience in erosion control and landscape restoration studies (Yaltırık, 1993). Although most willow species adapt to an oxygen-free environment, they prefer more mineral-rich and organic soils. Usually their blooms are in the form of catkins and they appear before the leaves or with the leaves. In addition, willows are the cheapest woody plants, but they have the ability to provide a powerful landscape view and instant visual impact to industrial parks (Bennett, 1999).

2. DIFFERENT USES OF WILLOWS

The main features affecting the use of willows in environmental repair works are that they are both for repair and improvement in landscaping and that they have a very different and wide range of uses. These features are as follows:

- **In Landscape Arrangement Studies:** Willows can be used as a pioneer plant in landscape restoration work in conditions of lack of nutrients and oxygen (Kuzovkina & Quigley, 2005). The willow species mentioned above are suitable for use in landscape arrangements to create a green belt, as they are content with their ecological needs in the growing environment, easy to grow and grow fast. Especially as an ornamental plant of the *Salix aegyptiaca* (Musk Willow) type, it is suitable for use in recreation areas due to its decorative nature and the beautiful scent of its flowers.
- **Water and Wind Erosion:** *Salix* L. species are often planted on the banks of rivers, lakes, artificial ponds, drainages and natural channels to stabilize natural channels, as well as their fibrous roots minimize erosion with a continuous vegetation to provide

mechanical stabilization on slopes (Morgan & Rickson, 1995; McCreary & Tecklin, 2000; Lefkowitz, 2002).

- **Ecological Restoration, Wetland and Wildlife Protection:** Willows are species that provide rich habitat and food for different creatures, and provide shelter and shade for animals. Willow communities create opportunities to view songbirds and water birds in addition to their recreational value (Sommerville, 1992; Hightshoe, 1998).
- **Land Reclamation:** Willows are characterized by increased air concentration and soil pollutants in both actively working and abandoned industrial areas (Zvereva et al., 1997).
- **Phytoremediation:** The plant's high water consumption rates due to its superior growth and biomass production and efficient hydraulic control of soil water levels make willows suitable as a potential "bioreactor" for cyanide removal (Kuzovkina & Quigley, 2005; Yousefi, 2019).
- **In the Field of Medicine:** Antioxidant, anxiolytic and hypocholesterolemic effects have been shown in traditional medicine as a remedy for the relief of anemia, dizziness, heart tonic substance (Karimi et al., 2011; Asgarpanah, 2012).
- **Wind screen:** It can be used for noise retention on highways, willow fences along concrete walls, building solid soil-filled walls. Strengthens the improvement of the environment while suppressing noise, dust and exhaust (Bache & MacAskill, 1984).
- **Biomass Production:** Willow appears to be among the most promising biomass fuels in many countries. Willow grows faster

with more carbon removal than softwoods with seasonal growth (Vandenhove et al., 2001; Lamlom & Savidge, 2003).

- **In the food industry:** *Salix aegyptiaca* is known as "Bidmeshk" in Iran and is grown in many places, especially in Urmia, in the North West of Iran. The aqueous extract and essential oil of its flowers are also used in the preparation of delicious syrups and especially the local Confectionery that name is Noghl-e Urmiye (Karimi et al., 2011; Rabbani et al., 2011).
- **Cut Flower:** Since *Salix aegyptiaca* flowers are scented, they are used especially in cut floristry in Europe. The flowers on the branches of this plant, which started to bloom in the early spring, are quite impressive, and these flowering branches are brought in bunches and offered for sale. It is a highly preferred plant in cut floriculture due to its beautiful scents, beautiful images and long vase life.
- **Perfumery:** Because of its fragrant flowers, *Salix aegyptiaca* var longifrons Bronm is used in the preparation of perfume additives (Yousefi, 2019).

3. SOME TYPES OF WILLOW THAT CAN BE USED IN LANDSCAPE REPAIR WORKS

Studies have shown that the use of natural plants in erosion prevention, land reclamation and landscape restoration works is always a reason for preference as they adapt to the existing ecological conditions. Willow plant is preferred in erosion prevention and landscape repair works as a good soil catcher in keeping the slopes, due to its rapid development, its ability to have a continuous fibrous root layer that can easily root, and its ability to develop very wide and common roots (Polunin, 1976; Morgan & Rickson, 1995; Kuzovkina & Quigley, 2005; Anonymous, 2020). Some willow species that can be used in landscape restoration works are given below. These willow species are used in a sustainable approach, especially in areas that are idle and not subject to any use and in areas where landscape restoration is needed, if ecologically appropriate. In addition, these areas become a center of attraction for the people and visitors living in the vicinity, providing an ecological approach to use.

3.1. *Salix aegyptiaca* (Musk Willow)

It spreads naturally in Northern Iraq, Iran, Afghanistan and Syria. South Eastern Anatolia in Turkey, Bitlis, Van and Hakkari occur naturally in the provinces. *Salix aegyptiaca*, an Irano-Turanian phytogeographic element, shows a vertical distribution area and grows naturally at altitudes ranging from 2700 m to areas close to sea level (Skvortsov & Edmondson, 1982; Avcı, 1999). *Salix aegyptiaca* species are tall shrubs and trees that can grow up to 2.5-10 m (Figure 1a). Flowering time is in early spring and its flowers are fragrant and gray in color. It tolerates most of the soil pH and wet soils (Zargari, 1988; Asgarpanah, 2012). It can be used effectively in water and soil protection, Phytoremediation (Glass, 1999) and erosion control. It spreads in regions with an annual average temperature of 4 °C (Avcı, 1999; Yousefi, 2019). It can be used as an ornamental plant in green areas, parks and gardens. It can be used in green areas, parks and gardens as an ornamental plant.

3.2. *Salix fragilis* (Crack willow)

Turkey, which spread in Europe and creeks between 900-1600 m altitudes in Siberia is a plant with Euro-Siberian phytogeographic elements. Istanbul in Anatolia East and North Anatolia in Turkey, Kastamonu, Bolu, Gumushane, Kars, Erzincan, Elazığ, Erzurum and Ağrı are naturally present in the provinces (Eminağaoğlu et al., 2014). It is a tree up to 20-30 m in height (Figure 1b). Flowering time is between April-May. It makes the best development in deep soils with poor drainage. But he wants plenty of sun in heavy soils. The plant, which grows well in calcareous soils, is the most suitable for the dry temperate zone as it grows collectively on steep slopes and areas prone to erosion (Rawat, 2006).

3.3. *Salix matsudana* (Chinese willow)

Its homeland is North China, Manchuria and Korea. It is generally planted in these areas (Eminağaoğlu et al., 2014). Temperature and humidity requirements are variable. It is resistant to cold weather conditions. It is the most drought resistant type among willows. It can also grow in heavy clay, acidic, damp, wet and sandy soils. It is resistant to dirty weather conditions and wind. It is a densely branched tree that can grow up to 10-12 m (Figure 1c). Roots are predominantly dispersed in 0-100 cm soil layer (Peng et al., 2015). In recent years, it has been grown as an ornamental plant in parks and gardens and is an important ornamental plant with its landscape restorative feature.

3.4. *Salix caprea* (Goat willow)

Turkey, in opening plants spread naturally in the forests in Europe and North Asia, the creek and the water's edge, peat lands and grasslands in sea level at an altitude of 2300 m can be grown naturally. The plant, which is a European-Siberian phytogeographical element, has a natural distribution in Turkey in Rize, Artvin, Kırklareli, Istanbul, Bursa, Bolu, Zonguldak, Ordu, Gümüşhane, Rize, Kars, Balıkesir, Erzincan, Erzurum and Bitlis provinces (Eminağaoğlu et al., 2014). It is a pioneer tree in forest areas, barren lands and gravel areas. It is in the form of a tall shrub or a small tree 3-10 m tall (Figure 1d). Although it can grow outside of stream vegetation, it develops well in wet, neutral and lime-rich soils. Resistant to polluted air and wind, the plant especially likes sunny areas. It is resistant to winter and sea cold, the remarkable catkins-shaped flowers of this species appears in March-April and these flowers are used in beekeeping. It is preferred in landscape repair works and border afforestation as a good soil retainer in keeping the slopes, as it has the ability to easily root and develop very wide and common roots (Avcı 1999; Küçükgöksel, 2010). This plant species also attracts attention with its aesthetic appearance and is frequently used in open green areas, parks and gardens.

3.5. *Salix elbursensis* Boiss.

Iran, Turkey and Northern naturally occurring in plants, can grow naturally in 1200-2600 m altitude. It is a species with a European-Siberian phytogeographic element along rivers. North Eastern and Eastern Anatolia in Turkey Gumushane, Bayburt, Kars, Malatya, Erzurum, Van, Siirt, Sirnak and Hakkari provinces found naturally (Eminağaoğlu et al., 2014). The branches of the plant are long, flexible branched and have light brown-dark purple shoots. It is in the form of a shrub with hairless buds or a small tree 2-5 m long (Figure 1e), (Avcı 1999). This plant species is used for soil improvement of open spaces and to reduce the toxic effects of environmental pollutants (Malá et al., 2010). This plant is suitable for use in landscape repair work.

3.6. *Salix viminalis* L. (Basket willow)

It is a species with a European-Siberian phytogeographic element that spreads on riversides and flat lands in Europe, Crimea, North and East Asia and the Himalayas. It is one of the types used in basket making because its branches are very flexible (Eminağaoğlu et al., 2014). For this reason, it is artificially grown a lot. It is in the form of an upright shrub or a small tree that can grow 3-5 m in length. Can be used for cold resistant, soil rehabilitation and reducing the toxic effects of environmental pollutants (Lebrun et al., 2019). This type is widely used in large parks, gardens and areas requiring repair (Figure 1f).

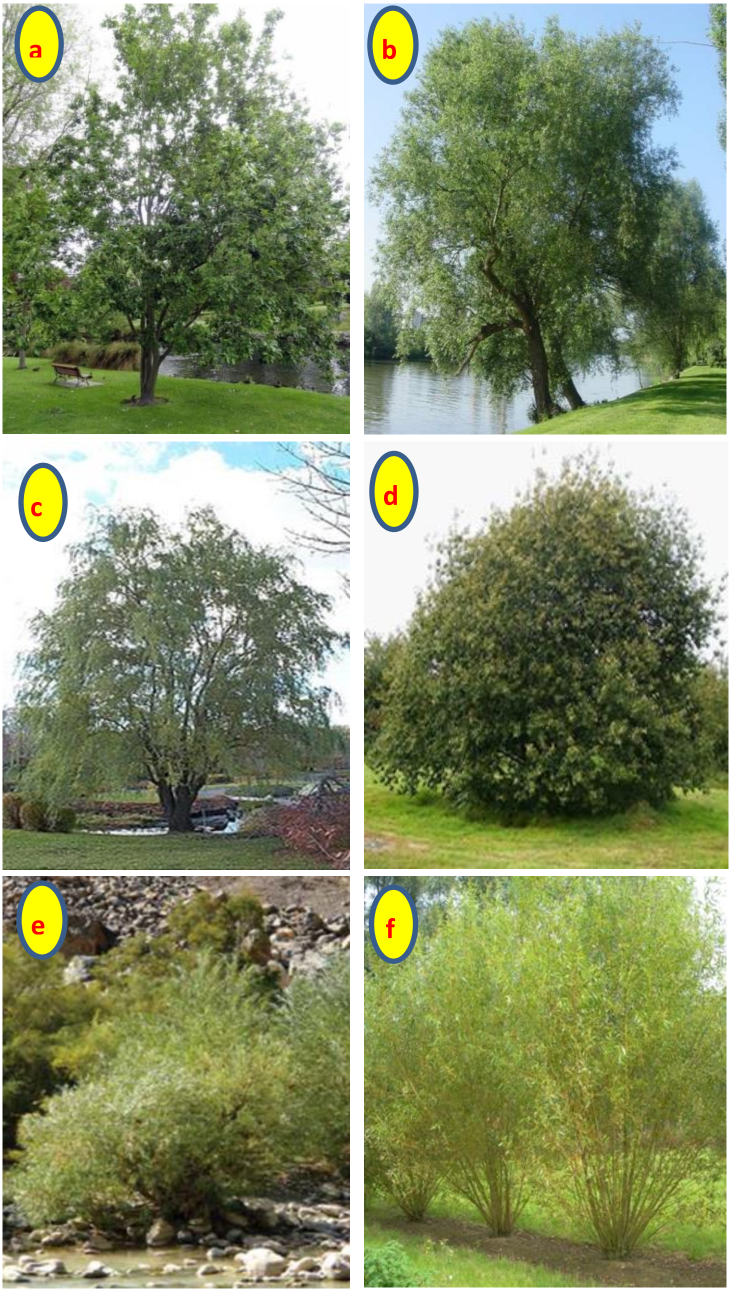

Figure 1: Natural growing willow species that can be used in landscape restoration works, **a:** *S. aegyptiaca*, (URL-1), **b:** *S. fragilis* (URL-2). **c:** *S. matsudana* (URL-3), **d:** *S. caprea* (URL-4), **e:** *S. elbursensis,* **f:** *S. viminalis* (URL-5)

CONCLUSION

Some ornamental plants are also used in restoration works, soil reclamation and erosion prevention studies besides landscape design. In this context, willows (*Salix* L.) are plants that are frequently used in restoration works, with their ability to quickly root, develop and hold the soil tightly with its wide root system. These plants are particularly suitable for fixing around river banks, lakes, artificial ponds, drainage areas and natural channels for restoration purposes. Willows gain importance as the main element of landscape restoration and restoration projects due to their important ecological relations and functions in wetlands. For this reason, in this study, taking into account the benefits of natural plants in green space planning, it is recommended that willows, which are naturally grown, are used in idle empty areas that need landscape restoration with a protection and sustainable approach. These areas can turn into a more visually and physically useful and aesthetically pleasing landscape function. In this respect, the benefits that will occur in the case of the use of willows, which are prominent in this study, especially that grow naturally in the world, are listed below.

- **Increasing diversity in the green field:** Due to the compatibility and abilities of natural willows (*Salix* L.), using it as an ornamental plant in the green field will double the variety of species and ecotypes (in terms of color, shape, size and harmony) and increase the success rate of design and design flexibility. While the presence of natural plants in green areas is a sign of the climatic and cultural potential of the region, the use of a number of non-native species in the green area causes the fragility of the built ecosystems.

- **Protection of natural plants in terms of biodiversity:** Willows (*Salix* L.) a natural plant species, will find use in many areas such as landscape design and restoration if the threat of extinction is eliminated. In this respect, the sustainability and protection of this plant in terms of biodiversity is very important.
- **Cost reduction:** Generally, creating technical and managerial staff in green areas and allocating quality soil and water resources to green areas are costly operations. Part of the high cost can be recycled by growing natural willows (*Salix* L.) in the green field. Natural willows generally save on irrigation water consumption because they need less water than non-natural plants.
- **Achieving educational and research objectives:** Considering the long history of the use of natural willows (*Salix* L.), this plant, which grows naturally in many countries, is unfortunately not used in education and research. In this respect, institutes, institutions and organizations and experts working on plant sciences, agriculture and natural resources should work in coordination to increase social awareness on these issues and this knowledge and experience should be accessible.

With this study, it is planned to create a resource that will lead similar practices to be widespread by relevant stakeholders in our country. In this way, the importance of the applications for willow (*Salix* L.), which is widely used in many areas, especially in landscape restoration works, and which is naturally spread in many countries around the world, will once again be demonstrated, especially in landscape function. One of the important issues in the planning work to be done is to pay attention to planting willows at long distances from settlements and historical artifacts. Otherwise, good and fast growing willows can damage buildings and historical buildings whose roots are underground. As a result of this study, where the use of natural willows in sustainable environment and landscape restoration works is recommended, it will be possible to protect natural habitat and biodiversity in the areas where it is applied. These habitats, which are formed by natural processes, will have an important potential for the organization of urban ecological green areas, an aesthetic and functional structure with landscape design studies and finally, they will have the ability to create beneficial results with their ecological and economic functions.

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CHAPTER 15

EVALUATION OF PHYTOCHEMICAL COMPOUNDS, BIOLOGICAL ACTIVITY AND USES IN ETHNOBOTANY OF GENERA IN TURKEY OF LILIACEAE FAMILY USED AS ORNAMENTAL PLANTS

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INTRODUCTION

In the perception of beauty in human consciousness, the senses significantly affect the cultural attitude towards objects (Alp et al., 2020). Plants have the capacity to create a visual perception of beauty, especially due to their eye-catching colors. In addition to this statement, maintaining the connection between human and nature, and providing biological comfort, increasing people's productivity, also eliminates negative emotions (Öztürk & Acar, 2019). It is stated in the literature that plants were cultivated and grown during the settled life (Richards, 2001; Perry et al., 2008; Alp et al., 2020). The cultivation of plants has been the most important development in the formation of the agricultural sector in human history. The rapid increase in the population in the 18th and 19th centuries made it more difficult to meet the food need, the rapid increase in urbanization due to the rise of migration to the cities in the villages after the 1950s, the longing for nature of the people who moved away from nature due to the positive contribution of plants to human psychology and has revealed the necessity to mask this longing by producing, selling or planting plants under certain conditions (Nemutlu Erduran, 2013; Öztürk & Acar, 2019; Alp et al., 2020). In general, the plants produced and sold to have an aesthetic appearance, be functional, and provide economic gain are called ornamental plants (Nemutlu Erduran, 2013). It is divided into four subgroups: ornamental plants, indoor, outdoor, cut flowers, and bulbs (Ay, 2009; Nemutlu Erduran, 2013). Potted and cut flower production in Turkey increases every year, and exports to domestic and other countries are common. Many different types of exotic plants are produced in potted and cut flower production (Özdemir & Çiçek, 2017). Outdoor ornamental plants reduce air pollution, masking of noise, and aesthetic appearance while determining the transportation axes and improvement of climatic conditions. It is used in parks and gardens, green areas, roads, rural areas, and recreation areas. Many plants in nature are researched and added to the literature as outdoor ornamental plants (Ergun, 2005; Yılmaz, 2006). Güner et al. (2012) stated that 11707 plant taxa in Turkey stated that 3649 taxa are endemic. This number is very close to the number of plant taxa found in the whole European continent (Dilaver et al., 2020). According to the AIPH Statistical Yearbook (2018), the rate of Asia/Pacific (73.85 %) in the production area of 650000 ha of cut flowers and potted plants in the world is approximately three times higher than the total of the rest (26.15 %). Countries such as Colombia, Ecuador, Ethiopia, and Kenya have become important exporters due to the low labor force and favorable climatic conditions, besides the traditional production countries such as the USA, Japan, and the Netherlands, especially in cut flowers and potted plants.

In order to be used in introducing and landscaping of geophyte flora species found in Turkey, many geophyte gardens have been established, and the most striking of them is the geophyte garden in Yalova in Turkey. These geophytes, which can be used in landscaping, park and garden arrangements, have started to attract more attention as ornamental plants with their advantages such as being resistant to the underground parts of their bodies and being less affected by adverse environmental conditions. It is mostly preferred in parks and gardens' arrangement due to the different seasons when geophyte species bloom and their showy flowers (Seyidoğlu, 2009). Liliaceae family has beautiful plant structures and flowers in geophytes. It attracts attention all over the world, especially in the ornamental plants' sector. This family, which is so close to humans, is frequently included in ethnobotany and scientists' studies. Liliaceae family, which has an important place as ornamental plants, is an attractive ornamental plant. It is also used in the ethnobotanical field and biological studies with these species. In this review article, studies of these species have been investigated and evaluated.

1. THE PLACE AND IMPORTANCE OF GEOPHYTES IN THE ORNAMENTAL PLANT SECTOR

Geophytes have an important place in ensuring plant diversity, advancing the ornamental plant sector, and growing economically. The term geophyte is the general name given to plants with bulbous, rhizome, and tuberous stems that come together, spend specific periods of the year under the soil, and when the growth is completed, the above-ground part withers and falls, but the underground part can continue to live. While flower and leaf development co-occurs in some geophyte plant species, some species occur at different times (Haspolat et al., 2016; Elias et al., 2018; Turan, 2020). In addition to being beautifully flowered in general, geophytes are actively used to produce perfumes from the etheric oils they contain and the pharmaceutical industry with many chemicals they include (Nasırcılar & Karagüzel, 2006). Since this situation attracts foreigners' attention, many types of geophytes have been exported from their natural environment for about a hundred years. It is in danger of extinction due to unconscious dismantling and because many species have long periods of new shallot formation in the natural environment (Ekim et al., 2000; Nasırcılar & Karagüzel, 2006). In addition, rapid population growth, urbanization, excessive and unconscious grazing, field opening, road building or widening, tourism activities, forest fires, etc., puts natural populations in danger of destruction and extinction (Haspolat et al., 2016). Nowadays, especially wild plant and animal species that are endangered in order to Turkey in 1996 to protect plant species under threat agreement governing international trade (CITES) has become a member and took control of the export of these species (Ekim et al., 2000; Nasırcılar & Karagüzel, 2006; Karagüzel et al., 2007).

Geophytes are highly preferred in the ornamental plant sector due to the beautiful flowers they have, and some varieties of geophytes bloom in every season. Geophyte bulbs have an important place in the ornamental plants' sector in terms of contribution to some countries of the world and Turkey's economy. However, while only some of the geophytes can be reproduced with tissue culture in our country, most of them are removed from nature and exported. Many of these species are used in diversification activities abroad, especially in our country. Most of the bulbs removed with or without permission are exported to the Netherlands and sold to other countries from the Netherlands to be used in landscaping and gardening. Most of the exports of geophyte species are exported to the Netherlands. It is also exported to many countries such as Germany, England, Japan, Denmark, but more than the value of the import value than the export value of ornamental plants, and Turkey is dependent on external accordingly. The most important geophytes genera exported are *Cyclamen* L., *Galanthus* L., *Leucojum* L., *Anemone* L., *Eranthus* G. Don, *Fritillaria* L., *Lilium* L., *Tulipa* L. (Haspolat et al., 2016). According to 2019 data in TUIK (Turkish Statistical Institute) (TUIK, 2019), bulbs planted area decreased from 2013 to 2019 year in Turkey decreased by 140625 m^2. While 33012460 units were produced in 2013, this number is constantly decreasing until 2018, but in 2019 alone, it was nearly twice as high as in 2013 (62537229 units) (Table 1).

Table 1: Between change the years 2013-2019 of total production area and produced quantity in Turkey (TUIK, 2019)

Year	Planted Area (m^2)	Produced Quantity (unit)
2013	552770	33012460
2014	567505	30059530
2015	612585	27200330
2016	597305	25337330
2017	426885	21833825
2018	493930	88657000
2019	412145	62537229

2. STUDIES WITH LILIACEAE FAMILY

Liliaceae family is represented by 18 genera, 746 species globally, and 5 genera, 91 species in Turkey (Güner et al., 2012; Plantlist, 2020). Studies on the Liliaceae family have shown that it is mainly of Eurasian origin and that its genera are about two-thirds in the old world, and the rest are in the new world (Vinnersten & Bremer, 2001; Patterson &

Givnishi, 2002; Allen et al., 2003). The species belonging to the Liliaceae family are bulbous species. They have an underground organ that consists of folding of thick fleshy scales containing nutrients on top of each other, containing growth cone, leaf, and flower bud in development. Bulbs can be shelled like *Tulipa* and *Narcissus* L. or shelled like *Fritillaria* (Mammadov et al., 2017).

2.1. Biological Activity Studies with *Erythronium* Genus

Erythronium L. genus is represented by 29 species in the world and only one in Turkey (Güner et al., 2012; Plantlist, 2020). *Erythronium* is widely distributed in temperate forests in Eastern North America, Western North America, East Asia, Central Asia, and Europe (Allen et al., 2003). *Erythronium sibiricum* (Fisch. & C. A. Mey.) Krylov species is used in ethnobotany in China, and *E. sibiricum* bulbs are boiled in goat's milk or millet and used for waist and knee pain in Kazakhistan (Chen et al., 2016). *Erythronium japonicum* Decne. is used in ethnobotanics in China, Japan, and Korea and is eaten for stomach and digestive disorders (Lee et al., 1994; Lee et al., 2012). *Erythronium grandiflorum* Pursh is boiled with milk or broth by Tatars (Allen et al., 2003).

The biological activity of the polysaccharides of *E. sibiricum* bulb was studied by Chen et al. (2016), and DPPH, ABTS, and hydroxyl radicals were tested against ascorbic acid. Very low activity was observed compared to ascorbic acid but in the anti-inflammatory and analgesic activities experiment on ear edema in mice, it was found to be statistically active compared to the control group. Kasimu et al. (2017) isolated and purified the water-soluble polysaccharide (ESBP2-1) of the *E. sibiricum* bulb. It was found that ESBP2-1 of the weight of purified 9.4×10^5 Da consisted of glucose, galactose, and arabinose in the proportion of 24.3:1.1:1 and thus a glucan containing a small amount of non-glucose residues. The macrophage cell plays an important role in host defense and innate immune response. Activation of macrophages is a self-defense mechanism that protects the host against pathogen infection. The effect of the obtained ESBP2-1 on RAW 264.7 cells was studied for 24-72 hours to observe the effect on macrophage proliferation. It was found that it stimulated the RAW 264.7 further proliferation after 48 and 72 hours. 48 and 72 hours results were similar (Wang et al., 2013). The NO change was measured in the macrophage culture of the RAW 264.7 cells of ESBP2-1 at concentrations of 100, 200, and 400 mg/mL, and the values of 6.73, 7.41, and 10.75 M were found, respectively, and NO changes were found to increase in the macrophage culture. ESBP2-1's stimulation of RAW 264.7 cells shows that it helps in strengthening the immune system. Boo et al. (2013)'s biological activity study of methanol extracts of *Salicornia herbacea* L., *E. japonicum,* and *Phragmites communis* Trin. species, *E. japonicum* had 66.5 % rate in α-amylase inhibitory activity test, while the highest inhibitory activity was found in the root of *P. communis* with 69.3 %. According to the article, it was found that extracts of *S. herbacea*, *E. japonicum,* and *P. communis* act effectively as α-amylase inhibitors leading to a reduction in starch hydrolysis and thus lowering glucose levels. In the same study, the DPPH antioxidant activity of the leaf, root, and flower parts of *E. japonicum* species was evaluated. It was found that the leaf part was more active than the others with a value of 2.2 ± 0.13 at 250 mg/L. Lee et al. (2012) isolated erthrojaponiside of megastigmane glucoside (Figure 1.1) together with six known megastigmane derivatives (Figure 1.2-7) as a new compound from the methanol extract of aerial parts of *E. japonicum* species.

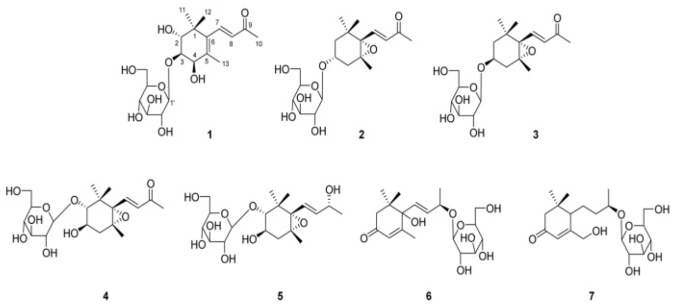


Figure 1: Erthrojaponiside (**1**), Euodionoside A (**2**), Icariside B_2 (**3**), 3*β*-hydroxy-5*α*,6*α*-epoxy-*β*-ionone-2*α*-*O*-D-glucopyranoside (**4**), (2*R*,3*R*,5*R*,6*S*,9*R*)-3-hydroxy-5,6-epoxy-*β*-ionol-2-*O*-*β*-D-glucopyranoside (**5**), (6*R*,9*R*)-3-oxo-*α*-ionol-9-*O*-*β*-D-glucopyranoside (**6**), (6*R*,9*S*)-megastigman-4-en-3-one-9,13-diol-9-*O*-glucopyranoside (**7**) (Lee et al., 2012)

Isolated α-Methylene butyrolactone (Tulipalin A) from *E. grandiflorum* by Diamond et al. (1985) and from *Erythronium americanum* Ker Gawl. by Cavallito & Haskell (1946). Christensen & Kristiansen (1995) was reported that Tuliposide A, the precursor of allergenic Tulipalin A, appears to be widely distributed in the genera *Alstroemeria*, *Erythronium,* and *Tulipa* (Figure 2). In the anticancer study of *E. japonicum* extract on ICR mouse cells with induced abdominal cancer and L1210 cells, the number of L1210 cells were decreased after 3 days of culture, and the little effect was observed against normal lymphocytes (Shin et al., 2004). In the cytotoxicity activity study of methanol extracts of the above-ground part of 11 Korean medicinal salad plants against HCT-116, *E. japonicum* extract, IC_{50} value was found to be 44.06 µg / mL, and *Petasites japonicus* (Siebold & Zucc.) Maxim. species was found with the highest activity <25.0 µg / mL, IC_{50} value (Heo et al., 2007).

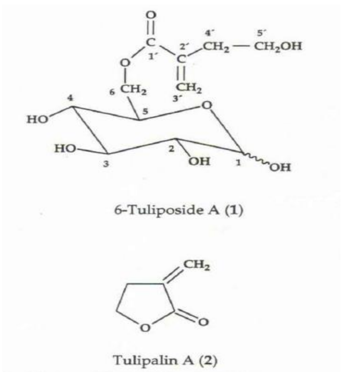


Figure 2: Chemical structures of 6-Tuliposide A (1) and Tulipalin A (2) (Christensen & Kristiansen, 1995)

In the same study, the DPPH antioxidant activity of *E. japonicum* species was 1.0 ± 0.07 mg/100g, IC_{50} value at 6.3 mg/100g concentration. The research investigating the anti-inflammatory effects and mechanisms of *E. japonicum* aqueous extract, it was shown that it suppresses lipopolysaccharide (LPS) induced inducible nitric oxide synthase, cyclooxygenase-2, ionized calcium-binding adapter molecule 1, and pro-inflammatory cytokines in BV2 microglial cells. In addition, by inhibiting p38 and c-Jun NH_2 terminal protein kinase of *E. japonicum* and inhibiting ERK/Nrf2/HO-1 (extracellular signal-regulated kinase/Nuclear factor erythroid 2-related factor 2/heme oxygenase-1) signal in BV2 microglia cells. It has been shown that it can relieve inflammatory pain through suppressing NF-kB (Park & Kim, 2020). *E. japonicum* and *Corylopsis coreana* Uyeki extracts treated with 1,3-DCP (1,3-Dichloro-2-propanol is a widely used pesticide and a well-known food contaminant produced during various food manufacturing, which is suggested to have toxicological properties). Its protective effects against cells have also been investigated, and 1,3-DCP has been found to stimulate the expression and catalytic activity of cytochrome P450 2E1 (CYP2E1). This enzyme produces reactive oxygen species in the liver. However, co-treatment of 1,3-DCP with extracts significantly reduced ROS formation and inhibited CYP2E1 activity without affecting its expression. Consequently, it induced oxidative stress through the high level of CYP2E1 inhibited by the extracts, protecting cells against the effects of 1,3-DCP (Boo et al., 2012). Anti-asthmatic effects of an extract of *E. japonicum* in a mouse model of ovalbumin-induced asthma have been studied and found that it attenuates asthma-related morphological changes in mouse lung tissue (Seo et al., 2016). In Moon & Kim (2012) study, β-sitosterol, 3-O-β-D-glucoside were isolated from *E. japonicum* bulbs, and campesterol, stigmasterol, palmitic acid, stearic acid, oleic acid, arachidic acid, behenic acid, tricosanoic acid, lignoceric acid were identified.

2.2. Biological Activity Studies with *Fritillaria* Genus

Fritillaria L. genus is represented by 141 species in the world and 35 species in Turkey (Güner et al., 2012; Plantlist, 2020). The rate of endemism in the Turkey *Fritillaria* genus of 36.53 %. This high rate of this genus flora of Turkey shows that it can be the center of genetic diversity. Besides, species belonging to the genus *Fritillaria* are of increased agricultural and economic importance. The species belonging to the genus *Fritillaria* show a wide variation in their morphological characteristics and physiological adaptation to the environment. As a result of this variation, they are widely used as bulbous ornamental plants (Tekşen & Aytaç, 2012). It is known that bulbs belonging to the genus *Fritillaria* have been used in traditional Chinese medicine for thousands of years as an important drug against cough, expectorant, and high blood pressure (Wang et al., 2006; Kocoglu et al., 2018). *Fritillaria pallidiflora* Schrenk and *Fritillaria cirrhosis* Boiss. are used in Chinese medicine to treat cough suppressant, asthmatic, and expectorant (Wang et al., 2006). *Fritillaria anhuiensis* S. C. Chen & S. F. Yin is used in traditional medicine in China to treat cough, asthma, and bronchitis (Li, 1995). *Fritillaria acmopetala* subsp. *wendelboi* bulbs are used to headache and treat rheumatism in Turkey (Ozturk & Altay, 2017). It is stated in Van-Turkey that *Fritillaria crassifolia* subsp. *kurdica* (Boiss. & Noë) Rix and *Fritillaria pinardii* Boiss. bulbs are used in wound healing treatment in traditional medicine (Mükemre et al., 2015). Chemical and pharmacological studies have shown that the alkaloids in bulbs prevent cough. Apart from alkaloids, it is also stated that different *Fritillaria* species contain components such as saponins, terpenoids, steroids, succinic acid, thymidine, adenosine (Kocoglu et al., 2018). In addition, some researchers emphasize that *Fritillaria usuriensis* Maxim. and *F. pinardii* bulbs are rich in calcium, iron, and zinc (Yukui et al., 2016; Özkaya et al., 2017). *Fritillaria* *thunbergii* Miq. bulbs and their flowers are known to contain plenty of vitamin E (XinHang et al., 2011). *Fritillaria delavayi* Franch. affects the energy pathways in the body, moisturizes the lung, and is primarily used to treat various lung diseases, including asthma, bronchitis, tuberculosis, and all cough types. It is also used to reduce swelling, nodules, goiter, and swollen lymph nodes. Again, these plant bulbs are known to destroy the fibrocystic areas in the breasts, and therefore they are used to treat breast cancer. Studies have shown that this herb especially reduces chronic prostate pain (Jin-xin, 2011). *Fritillaria roylei* Hook. bulbs are used against lung congestion, asthma, burns, stomach ailments, and tuberculosis in the Himalayan region-India (Kumar et al., 2020). Amiri & Joharchi (2013) reported that *Fritillaria imperialis* L. roots were used in the treatment of Joints Pain in Mashhad city, Northeastern Iran.

Extracts obtained from *Fritillaria* bulbs were tested against bacteria, and it was stated that *Fritillaria* had antibacterial properties on these bacteria. In addition, the antifungal effect of this herb is known (Maharjan et al., 2011; Kocoglu et al., 2018). Liu et al. (2012) *Fritillaria ussuriensis* isolated a water-soluble polysaccharide with a molecular weight of 4.1 x 104 Da, and DPPH antioxidant activity superoxide dismutase (SOD), glutathione peroxidase (GSHPx) enzyme activities were studied with these compounds. It was reported that it showed antioxidant activity by increasing the enzymatic and non-enzymatic antioxidant defense system. Polysaccharides consisting of glucose, galactose, arabinose, xylose, mannose, and rhamnose have been isolated and have been reported to have a strong antioxidant effect against DPPH, hydroxyl, and ABTS free radicals (Rozi et al., 2019). Shou et al. (2012) listed 27 compounds isolated from the *F. anhuiensis* species in the literature (Figure 3): peiminine **(1)**, verticine **(2)**, isoverticine **(3)**, ebeiedinone **(4)**, verticinedinone **(5)**, peimisine **(6)**, pengbeimine A **(7)**, pengbeimine B **(8)**, pengbeimine D **(9)**, 12R,15-dihydroxy-8(17),13(*E*)labdadien-19-oic acid **(10)**, 12S,15-dihydroxy-8(17),13(*E*)labdadien-19-oic acid **(11)**, 14(RS),15-dihydroxy-8(17),12(*E*)-labdadien-19-oic-acid **(12)**, 12R,13RS-dihydroxy-8(17),14-labdadien-19-oic-acid **(13)**, 8(17),12,14-labdantrien-18-ol **(14)**, *ent*-kauran-16β,17-diol **(15)**, *ent*-kauran-16α,17-diol **(16)**, *ent*-kauran-17-acetoxy-16β-ol **(17)**, *ent*-kauran-15-en-17-ol **(18)**, *ent*-kauran-15-en-3β,17-diol **(19)**, *ent*-kauran-3β-acetoxy-15-en-17-ol **(20)**, *ent*-kauran-3bacetoxy-16β,17-diol **(21)**, *ent*-kauran-16α-methoxy-17-ol **(22)**, cyclo-(Leu–Val) **(23)**, 3,7,11,15-tetramethylhexadeca-2*E*,6*E*,10*E*,14-tetraen-1-ol **(24)**, palmitic acid glyceryl ester **(25)**, bsitosterol **(26)**, daucostenine **(27)**.

Endophyte fungi living in *Fritillaria unibracteata* P. K. Hsiao & K. C. Hsia, gallic acid, rutin, and fluorizin were produced, which was exhibit an excellent antioxidant activity in the culture water (Pan et al., 2017). *F. roylei* was tissue cultured by *in vitro* method. The antioxidant activity of the pieces under tissue culture was studied. The highest DPPH antioxidant activity was found in the floral buds section with an IC_{50} value of 0.11 ± 0.01 mg/mL, and the lowest value in the callus section (2.71 ± 0.22 mg/mL, IC_{50}) (Kumar et al., 2020).

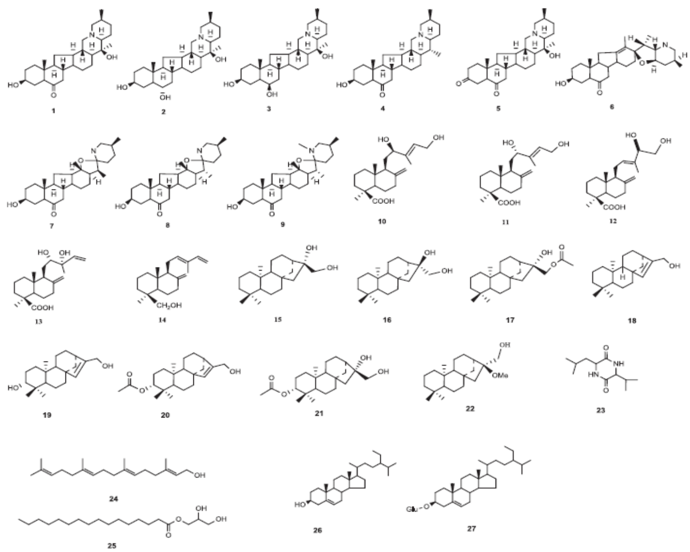


Figure 3: Structures of compounds isolated from *F. anhuiensis* (Shou et al., 2012)

2.3. Biological Activity Studies with *Gagea* Genus

Gagea Salisb. genus is represented by 209 species in the world and 31 species in Turkey (Güner et al., 2012; Plantlist, 2020). The above-ground part of *Gagea bohemica* (Zauschn.) Schult. & Schult.f., *Gagea chrysantha* (Jan) Schult. & Schult.f., *Gagea granatellii* (Parl.) Parl., and *Gagea villosa* var. *villosa* species are used as animal feed in Alaşehir-Manisa-Turkey (Sargın et al., 2013). Hives of bees are placed in places where *Gagea taurica* Steven and *Helleborus orientalis* Lam. are grown in Reşadiye-Tokat-Turkey (Yüzbaşıoğlu et al., 2020). *Gagea foliosa* (C.Presl) Schult. & Schult. f. bulbs are used in meals and salads in Sarıveliler-Karaman-Turkey (Bağcı et al., 2016). The underground part of *Gagea reticulata* (Pall.) Schult. & Schult. f. is consumed raw in Şanlıurfa-Turkey (Dağlı, 2015). It has been reported that the corm of

Gagea villosa (M.Bieb.) sweet is consumed raw after the outer shells have been peeled in Mardin-Turkey (Yeşil et al., 2019).

Antioxidant activity was studied with acetone, methanol, and water solvents of fresh and underground parts of *G. bohemica* and found the highest activity in acetone solvent of underground part and found an IC_{50} value of 0.652 ± 0.023 µg / mL for DPPH, 0.218 ± 0.0 µg / mL for ABTS. In the same study, 32 standard were used with UPLC-ESI-MS/MS analysis, and 2558.9 ppm of gallic acid was found in the major compound fresh part (Turan & Mammadov, 2020). In another study, among the ethanol and methanol extracts prepared from *Romulea ramiflora* Ten. and *Gagea fibrosa* (Desf.) Schult. & Schult. f. plants, the highest free radical scavenging activity in *G. fibrosa* was found the methanol extract (61.16 ± 1.4 %) of leaf parts (Mammadov et al., 2011). Özcan et al. (2018) studied the antioxidant activity, and High Pressure Liquid Chromatography (HPLC) analysis of 8 geophyte species and *G. granatellii* found the major compound (+)-catechin with a value of 33.72 ± 0.31 mg/100g as a result of HPLC, DPPH antioxidant activity was 10.50 ± 0.01 %, total phenolic content found 22.22 ± 0.00 mg GAE/100 g and *Crocus chrysanthus* (Herb.) Herb. was found to have the best DPPH antioxidant activity with 29.32 ± 0.01 %. The methanol extract of *Gagea granatelli* subsp. *granatelli* aerial part was found to have 5.62 ± 0.08 mg EAG/GE total phenolic content, 4.72 ± 0.14 mg EQ/GE total flavonoid content, and the DPPH antioxidant activity was found to be 220.49 ± 11.38 mg/mL, IC_{50} (Sofiane et al., 2017). In the same study, in the disc diffusion experiment against *Staphylococcus* *aureus*, *Escherichia coli, Bacillus subtilis* bacteria, it was stated that only *Escherichia coli* bacteria had an inhibition zone of 6.86 ± 0.17 mm.

2.4. Biological Activity Studies with *Tulipa* Genus

Tulipa L. genus is represented by 113 species in the world and 17 species in Turkey (Güner et al., 2012; Plantlist, 2020). According to 2019 data in TUIK (TUIK, 2019). from the years 2011 to 2019 in Turkey, it has increased the *Tulipa* genus's planted area to 142145 m^2. Accordingly, while 23732327 units were produced in 2011, this number increased to 40290500 units in 2019. *Tulipa cinnabarina* bulb is used in the treatment of abdominal pain in Turkey (Ozturk & Altay, 2017). In Karaman-Turkey, the *Tulipa humilis* bulb is used in meals and salads, while the *Tulipa cinnabarina* K. Perss. bulb is used to relieve abdominal pain (Bağcı et al., 2016). In Biga-Çanakkale-Turkey, the *Tulipa orphanidea* Boiss. ex Heldr. bulb part is cooked in ash (Kizilaslan Hançer et al., 2020). In Mardin-Turkey, *Tulipa agenensis* DC. leaves are mixed with honey or molasses and consumed to open the lung vessels (Eksik, 2020). *Tulipa edulis* (Miq.) Baker is used in Chinese medicine, treatment of sore throat, scrofula, ulcer, and postpartum blood stasis (Ma et al., 2014). In the Lesser Himalayas, *Tulipa stellata* var. *clusiana* fresh bulbs are peeled off and eaten raw as heart stimulants (Abbasi et al., 2015).

Youwei et al. (2008) studied crude aqueous extracts from 69 kinds of fresh flowers in southern China and DPPH, total phenolic content was measured. The DPPH antioxidant activity values of *Tulipa gesneriana*

L. (yellow) and *T. gesneriana* (red) species were 2.29 % and 2.93 %, respectively, total phenolic content values were found to be 0.46 mg CE/g and 0.348 mg CE/g. In the same study, the highest DPPH antioxidant activity was 71.261 %, and total phenolic content 17.882 mg CE/g value was found in *Rosa hybrida* cv. *samantha* (red). Sagdic et al. (2013) studied the cytotoxic and bioactive properties of *T. gesneriana* flowers with 5 different flower petal colors (claret red, orange-red, pink, violet, yellow), and the highest activity in the phosphomolybdenum reduction method was 48.69 ± 1.01 mg AAE/g dry extract and found total phenolic contents of 113.76 ± 0.27 mg GAE/g dry extract in orange-red color. Christensen (1999) 5 Tuliposide (6-tuliposide A **(1)**, 6-tuliposide B **(2)**, 1-tuliposide A **(3)**, tuliposide D **(4)**, tuliposide F **(5)**) identified and 2 new tuliposides (the lactonized aglycones tulipalin A **(6)** and (-)-tulipalin B **(7)**) were isolated from *Tulipa turkestanica* (Regel) Regel leaves, stems and flowers (Figure 4).

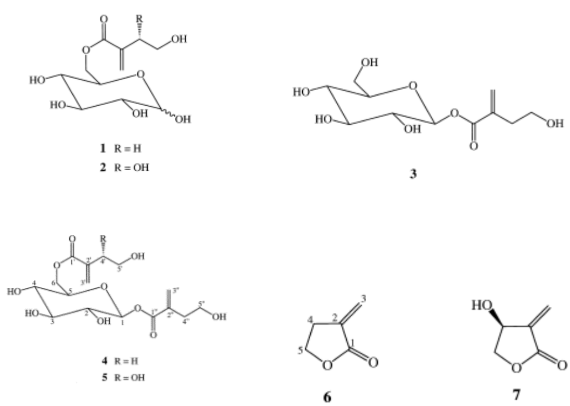


Figure 4: Structures of compounds from *Tulipa turkestanica* (Christensen, 1999)

Flowers of species belonging to the genus *Tulipa* (17 species and 25 varieties) were subjected to qualitative and relative quantitative investigation for anthocyanins. A total of five anthocyanins were identified as delphinidin 3-rutinoside **(1)**, cyanidin 3-rutinoside **(2)**, pelargonidin 3-rutinoside **(3)**, cyanidin 3-(2'''-acetylrutinoside) **(4)**, pelargonidin 3-(2''-acetylrutinoside) **(5)**. Pigments 1-5 showed 7 %, 43 %, 12 %, 2 % and 31 %, respectively (Torskangerpoll et al., 2005) (Figure 5).

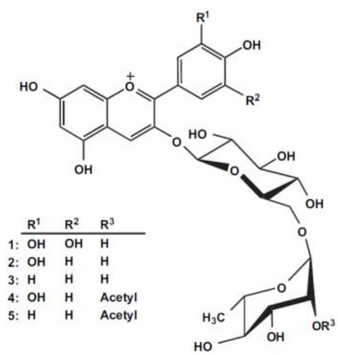


Figure 5: Structures of compounds from 42 species of *Tulipa* genus (Torskangerpoll et al., 2005)

Antibacterial effects of *T. gesneriana* petals at 10 % concentration against 7 bacteria (*Escherichia coli* 0157:H7, *Listeria monocytogenes* ATCC 7644, *Salmonella typhimurium* ATCC 14028, *Staphylococcus aureus* ATCC 25923, *Enterobacter cloacae* ATCC 13047, *Yersinia enterocolitica* ATCC 27729, *Pseudomonas aeruginosa* ATCC 27853) and found 9.8 ± 1.4 mm, 16.2 ± 3.0 mm, 9.0 ± 1.8 mm, 14.8 ± 3.1 mm, 7.2 ± 0.5 mm, 20.8 ± 2.6 mm, 12.3 ± 2.8 mm, 9.1 ± 2.4 mm, 7.3 ± 0.2 mm inhibition zone, respectively and has not observed any antibacterial effects on *Staphylococcus aureus* ATCC 29213, and *Escherichia coli* ATCC 23897 (Bayram et al., 2015). Ali-Shtayeh et al. (2014) found the highest activity in the flower part with the value of 64.41 ± 0.58 % in the DPPH antioxidant activity experiment performed in *Tulipa sharonensis* Dinsm. leaves, stem, and flower parts.

2.5. Biological Activity Studies with *Lilium* Genus

Lilium genus is represented by 111 species in the world and 7 species in Turkey (Güner et al., 2012; Plantlist, 2020). According to 2019 data in TUIK (TUIK, 2019) planted area of genus *Lilium* in Turkey between the years 2011-2019 decreased by 117161 m^2. Accordingly, while 12 614460 units were produced in 2011, this number decreased to 9282685 units in 2019. *Lilium candidum* L. is used to treat burns and swelling in ethnobotany, especially in Europe, so it was grown in monastery gardens in the Middle Ages (Mimaki et al., 1999). Historical records of the *Lilium* genus show that they were used to construct wreaths and palace gardens. *Lilium* species, which maintain their position as the most important ornamental plants, are aromatic plants and are used in many areas such as perfumery, medicine, and food fields (Mammadov et al., 2017). *Lilium monadelphum* M. Bieb. is used for softening, soothing, expectorant, and eczema treatment in Turkey (Yaldız et al., 2010). The flower of the *Lilium* genus in Çanakkale-Turkey is used to tighten the skin (Kökçü et al., 2015). Pieroni et al. (2005) stated that the *Lilium martagon* L. was used to treat liver disease in the Northern Albanian Alps. The bulb of *Lilium pyrenaicum* Gouan reduces abdominal hernia in Catalonia (Iberian Peninsula) (Rigat et al., 2007).

In Basque Country (Iberian Peninsula), roots of *L. candidum* are used in skin treatment (Menendez-Baceta, 2014). In Turgutlu-Manisa-Turkey, the above-ground part of *L. candidum* is used in Diuretic, cough treatment (Güler et al., 2015).

The natural components of jatropham-5-O-β-glucopyranoside and kaempferol-3,4',5,7-tetrahydroxy-flavone (Figure 6) isolated from *L. candidum.* In the eukaryotic test system established with *Hordeum vulgare* L. and human lymphocyte cells under *in vitro* conditions, cytotoxic and genotoxic activities as well as cell protection potential against radiomimetic zeocin were tested (Jovtchev et al., 2014).

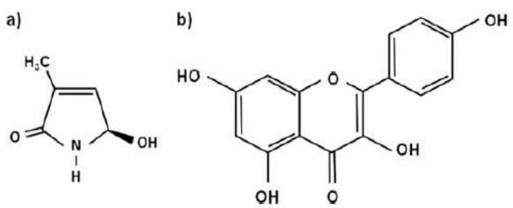
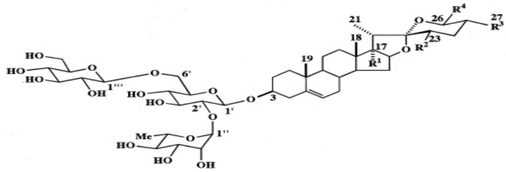
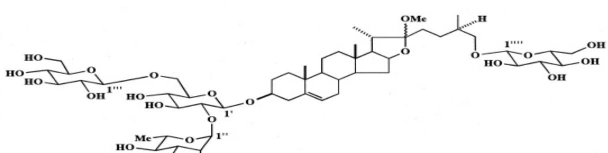


Figure 6: Chemical structures of jatrofam-5-O-β-glucopyranoside **(a)** and kaempferol-3,4 ', 5,7-tetrahydroxy-flavone **(b)** (Jovtchev et al., 2014)

In one study, the pollen grains of *L. martagon* were collected and exposed to exhaust gas for 5-10 days. The allergy potentials of the different pollen extracts were compared by skin testing and analyses of the blood eosinophil count and immunoglobulin E level in treated animals. Allergic bands were examined and controlled by the immunoblot method. The allergy test results examined show that pollen grains exposed to exhaust gas effectively stimulate allergic symptoms (Chehregani & Kouhkan, 2008). Five new spirostanol saponins (compounds 1-5) and new furostanol saponin (compound 6) were isolated from spring bulbs of *L. candidum*. Isolated saponins contain a branched triglyceride moiety such as *O*-α-L-rhamnopyranosyl-(1→2)-*O*-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranose with the formation of an O-glycosidic bound to C-3 of the aglycone as a common structural feature. The inhibitory activity of saponins in Na^+/K^+ATPase was evaluated, and 2.2 x10^{-5} M, IC_{50} in compound 1 and 4.7 x10^{-5} M, IC_{50} values were observed in compound no. 4, while no activity was observed in the others (Figure 7) (Mimaki et al., 1999).



	R^1	R^2	R^3	R^4
1	H	H	Me	H
2	H	H	CH_2OH	H
3	H	OH	Me	H
4	H	H	Me	OMe
5	OH	H	Me	OMe



6

Figure 7: Chemical structures of Spirostanol saponins **(1-5)**, Furostanol saponin **(6)** (Mimaki et al., 1999)

Lilium genus is used as edible and medicinal plant species in China. Potential sources of natural antioxidants of bulbs of 6 *Lilium* species unique to China (*Lilium regale* E. H. Wilson, *Lilium concolor* Salisb., *Lilium pumilum* Delile, *Lilium leucanthum* (Baker) Baker, *Lilium davidii* var. *unicolor* (Hoog) Cotton, and *Lilium lancifolium* Thunb.) were investigated according to their phenolic components and their nutritional antioxidant potential. *L. regale* (10381.49 ± 49.12 mgGAE/100g), *L. pumilum* (4177.39 ± 57.19 mgGAE/100g), *L. concolor* (3897.60 ± 42.54 mgGAE/100g), *L. lancifolium* (2827.25 ± 55.50 mgGAE/100g), *L. leucanthum* (2336.00 ± 29.28 mgGAE/100g), and *L. davidii* var. *unicolor* (2017.17 ± 140.20 mgGAE/100g) have the highest total phenolic content among these species, respectively. The results showed that bulb extracts exhibited strong antioxidant activity, showing positive correlation with total phenolic, total flavonoid and total flavanol contents. HPLC analysis revealed the major phenolic components rutin and kaempferol in extracts. Hierarchical team analysis showed that *L. regale* is in a group with high phenolic content and strong antioxidant activity. *L. leucanthum*, *L. davidii* var. *unicolor,* and *L. lancifolium* were grouped in the third group with their low phenolic contents and weak antioxidant capacity, while *L. concolor* and *L. pumilum* were grouped in a group characterized by proper phenolic content and antioxidant capacity. It has been suggested that *Lilium* bulbs can serve food and pharmaceutical applications as a potential source of natural antioxidants (Jin et al., 2012; Luo et al., 2012). The antioxidant activity of polysaccharides obtained from the *L. davidii* var. *unicolor* species has been studied, and it has been reported to have a good antioxidant activity (Zhao et al., 2013). Youwei et al. (2008) studied 61 plants collected from the southern China region. The polyphenolic content of *Lilium davidii* Duch. ex Elwes species was found to be 0.331 mg CE/g. The polyphenolic content of *Lilium brownii* F.E.Br. ex Miellez was found to be 0.287 mg CE/g, and the best result was observed *Rosa hybrida* E.H.L. Krause with 17.882 mg CE/g. The DPPH activity of the polysaccharide fraction (LP2-1) with a molecular weight of 8.52 x 103 kDa isolated from the species of *L. lanciflorum* was investigated, and it could not achieve a good result against BHT as synthetic antioxidant (Gao et al., 2015). Luo et al. (2012) identified 7 phenylpropanoid glycerides from *L. lancifolium* bulbs (Figure 8), and the antioxidant activity (DPPH) was performed was the best result with the 3rd fraction of 0.033 ± 0.001 (IC_{50} ± SD, mM).

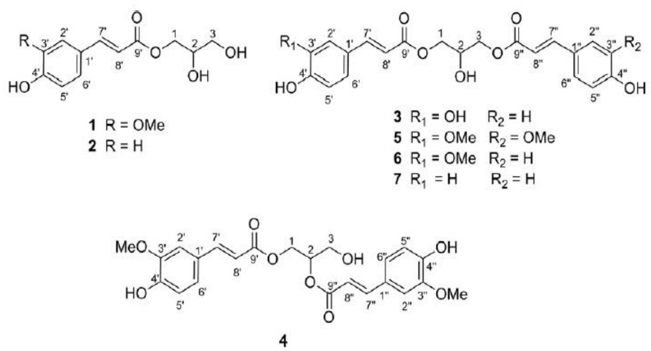


Figure 8: 1-*O*-feruloylglycerol **(1)**, 1-*O*-*p*-coumaroylglycerol **(2)**, 1-*O*-caffeoyl-3-*O*-*p*-coumaroylglycerol **(3)**, 1,2-*O*-diferuloylglycerol **(4)**, 1,3-*O*-diferuloylglycerol **(5)**, 1-*O*-feruloyl-3-*O*-*p*-coumaroylglycerol **(6)**, 1,3-*O*-di-*p*-coumaroylglycerol **(7)** (Luo et al., 2012)

As a result of HPLC analysis of *Lilium brownii* var. *viridulum* Baker with 14 standards, 127.0 ± 3.4 μg/g DW gallic acid, 22.8 ± 2.1 μg/g DW protocatechuic acid, 307.6 ± 4.1 μg/g DW chlorogenic acid, 267.9 ± 10.0 μg/g DW caffeic acid, 22.7 ± 0.2 μg/g DW p-coumaric acid, 335.5 ± 1.4 μg/g DW rutin was found (Xiong et al., 2014). Biological activity of *Lilium ledebourii* (Baker) Boiss. species was investigated, and 5.198 ± 0.17 mg GAE/g DW total phenolic content, 3.24 ± 0.52 mg/mL, IC_{50} DPPH antioxidant activity, 13.82 ± 0.74 μM/min superoxide dismutases (SOD) activity, 0.033 ± 0.002 μM/min peroxidase activity was found, catalase enzyme activity was not found (Ghanbari et al., 2018). Liang et al. (2018) studied 12 organs of the *L. pumilum* species, and the highest total phenolic content was found in the leaf part with 2490.5 ± 171.0 mg GAE/100g DW and total flavonoid content with 3069.1 ± 147.8 mg RE/100g DW.

CONCLUSION

The unconscious and wrong methods of removing geophytes from nature and selling them to other countries is a big problem, especially for plants with showy flowers, such as the genus of the Liliaceae family. In particular, tissue culture studies are continuing rapidly to prevent geophytes from being endangered and produced as ornamental plants. The fact that the Liliaceae family's species have beautiful and flamboyant structures has always attracted people's attention, and their effects on both ornamental plants and people's health have been investigated. In addition to the fact that the Liliaceae family's species have an important place in the ornamental plant sector in the world, their work in many large industries such as ethnobotanical, molecular biology, medicine, agriculture, and pharmacy is increasing day by day. Compounds belonging to many species belonging to the Liliaceae family have been isolated. Antioxidant studies conducted with extracts of these species or compounds that are isolated have been shown to show strong antioxidant activity showing positive correlation with total phenolic, total flavonoid, and total flavanol content of some species. It is also suggested that it can serve the food and pharmaceutical industry as a potential source of natural antioxidants.

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CHAPTER 16

DIFFERENT APPLICATIONS IN STERILIZATION OF THE NUTRIENT MEDİUM IN THE MICROPROPAGATION OF ORNAMENTAL PLANTS

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INTRODUCTION

Plant tissue culture is defined as the production of new tissue or secondary metabolites from plant parts such as cells, tissues, or organs under aseptic conditions, in an artificial nutrient medium. Application areas of tissue culture other than secondary products are obtaining disease-free plants, propagation of a large number of plants in a limited time (micropropagation), genetic development of plants (plant breeding), long-term conservation of plants, and transportation (Loberanth & Altman, 2010; Mohit, & Sirohi**,** 2018). Rapid propagation of high quality, disease-free plantlets in a short duration, and limited space is one of the most remarkable issues in the commercial field, and as a result, approximately two billion plants are now propagated by various tissue culture techniques. Ornamental plants are also the plant group where micropropagation is applied most intensively (Ahloowalia & Prakash, 2004). Among the potted ornamental plants, orchids, anthurium, spathiphyllum, and gerberas from cut flowers, which are the first group in this regard (Rout et al., 2006). However, in recent years, one of the subject that has been focused on micropropagation is the high production cost. Tissue culture is generally known as a method of high investment and operating expenses (Datta et al., 2017). In addition to the laboratory building and greenhouse installation costs, the investments made for the purchase of tools and equipment require a significant budget. Besides, high energy and labor costs which are called operating expenses, increase the unit cost of planting material. As a result of all these, the unit cost of tissue culture plantlets is higher than the plants propagated by conventional methods such as seed and cuttings (Ahloowalia, 2004; Savangikar, 2004; George & Manuel, 2013). Janes & Sluis (1991) reported that the plants obtained from tissue culture are 25 times more than those grown from seed and 2.5 times more than those grown from cuttings. The first step towards reducing the unit cost is to increase the number of shoots per explant (multiplication rate), as well as to reduce investment and operating costs. The use of different micropropagation techniques such as liquid media and bioreactor and in vitro autotrophic methods reduce costs and increase multiplication coefficient (Levin & Tanny, 2004; Fogaça et al., 2016).

One of the most important issues for a successful tissue culture is the selection of a suitable nutrient medium. There are quite a number of options for the nutrient medium. The nutrient medium contains different groups such as macro-micro nutrients, organic-inorganic compounds, vitamins, plant growth regulators, carbonhydrates, gelling agents (for semisolid medium) (Loberanth & Altman, 2010).

Besides the content of the nutrient medium, its sterility is the most essential feature. One of the critical milestones in the feasibility of plant tissue cultures is Louis Pasteur's (1822-1895) demonstration that microorganisms are responsible for the contamination and the principles of destroying them (Anonymous, 2020a). In this way, the sterilization of the nutrient media and the purification of microorganisms is fulfilled.

In conventional tissue culture studies, sterilization of nutrient media is performed with an autoclave. An autoclave is an equipment with high investment cost and high energy consumption. At the same time, its sterilization time is also long (Tiwari, et al., 2012; George & Manuel, 2013).

For these explained reasons, sterilization of the nutrient medium with autoclave is one of the processes that increase the unit cost of in vitro plantlets. As in all micro-propagated plants, low- cost options are investigated and implemented in also ornamental plants (Purohit et al., 2011; Datta et al., 2017). Therefore, alternative applications are required in the content and sterilization of the nutrient medium (Mvuria & Ombori, 2014; Biswas & Biswas, 2017). In this article, information is given about the methods used in the sterilization of the nutrient medium and the studies conducted with ornamental plants on this subject.

1. CONTAMINANTS IN TISSUE CULTURE

Fungi, bacteria, yeasts, viruses, viroids, mites and ants are factors that cause contamination in nutrient medium, laboratory air, bench surfaces and explants.

1.1. Fungi

Fungi enter the culture on the explants or as airborne spores, and causing contamination. It is usually the earliest symptom seen in culture. It is seen in different colors and shapes. The reasons of fungi contamination are the insufficient sterilization of the plant material (explant), errors made during culture and contaminated laboratory air.

1.2. Bacteria

Bacteria are one of the most difficult sources of contamination in tissue culture that can be prevented through sterilization. Bacteria may be controlled under in vitro conditions in some extent (Schmidt et al., 2004). They are usually found in the interior tissues of the tissue, so they cannot be eliminated by surface sterilization of the explant. Although some bacterial spores are on the explant surface, may remain unaffected by the normal sterilization procedure. Numerous bacteria such as *Agrobacterium, Bacillus, Corynebacterium, Enterobacter, Lactobacillus, Pseudomonas, Stephylococcus, Xanthomonas* have been found in tissue culture. They are detected in the appearance of a cloud around the explant or within the nutrient medium. They can be white, cream, pink, yellow in color.

1.3. Yeasts

Yeasts are found on the explant surface and in the air. From here, it enters the culture medium, causing contamination. Yeasts appear as ovoid bright particles and can exist as single cells or in in the form of chains or branches.

1.4. Viruses

Viruses and mycoplasma-like organisms are very small microorganisms, for this reason, it is very difficult to detect them.

Therefore, it is not always possible to state that tissue culture is pathogen-free. Meristem culture is the most effective method for eliminating viruses and similar organisms (Leifert & Cassells, 2001).

1.5. Micro-arthropods (Mites and Thrips)

They are insects that cause contamination after the initiation of culture. Mites feed on fungi, and due to their small size, are difficult to detect. The fungal infection line that reaches the nutrient medium from the side of the culture container is the symptom of the mite. It can be prevented by regular and careful cleaning of the laboratory environment and culture room.

Most of the contamination in tissue culture is caused by explant. Infections may occur as a result of insufficient sterilization of fungi and bacteria at the beginning of the culture. However, initially clean cultures can become infected even after a few subcultures. The reason for this, is endophytic bacteria in the tissue. As this type of infection spreads rapidly, the damage is greater and it becomes difficult to prevent. Other causes of contamination that are seen in the later stages of culture are insects such as mites and ants (Leifert & Cassells, 2001).

2. STERILIZATION METHODS

Sterilization methods applied in tissue culture are divided into two groups, physical and chemical methods.

2.1. Physical Methods

It is applied in three ways as high temperature, light (irradiation), and filtration. Physical methods include applications that are generally used in the sterilization of the laboratory air, sterilization of instruments and equipment used in inoculation, and sterilization of nutrient media. The most used applications among physical methods in tissue culture are dry heat (oven), pressurized steam high temperature (autoclave), flaming, filtration, and irradiation.

2.1.1. Dry Heat Sterilization Methods

2.1.1.1. Sterilization in A Dry Hot Air Oven

Sterilization takes place with dry heat. Electric ovens have thermostats and indicators and buttons for temperature and time adjustment. Since there is no moisture, it should be applied for a long time to ensure sterilization (Table 1). In the oven, tubes, petri dishes, forceps, scissors, scalpel, glass plates, filter papers, and other materials that do not deteriorate at high temperatures, are sterilized. The important advantage of dry heat sterilization is that it is safe for the environment and has no toxicity. Its disadvantages are the need for high temperature and electric energy (Dolapçı, 2016).

Table 1: Temperature and time required to be applied for glass material, forceps, scalpel in the oven (Dolapçı, 2016)

Temperature	Application time (h)
170 °C	1
160 °C	2
150 °C	2.5

2.1.1.2. Flaming

Another form of dry heat application. Generally, alcohol burners, or gas burner becs, are used for flaming. Sterilization of needles, forceps, the mouth of tubes, scalpel, and glass surfaces used during inoculation is conducted by passing through the flame (Arditti, 2008; Dolapçı, 2016). Burnt gas formation in sterile cabin is the disadvantage of this method.

2.1.1.3. Glass Bead Sterilizer

In this sterilizer, resistance raises the temperature of glass balls to a temperature of 250 °C. Sterilizes instruments such as forceps and scalpel. Sterilization duration is only 15-20 seconds.

2.1.1.4. Bacti-cinerator With Ceramic Core

The ceramic core element creates infrared heat. The temperature reached is 815.6 °C. Sterilization is completed in 5-7 seconds. Needle, tube mouth, scalpel, can be sterilized in this technical device. Since the instruments to be sterilized reach high temperatures in a short time, care should be taken against possible skin burns.

2.1.2. Pressurized Steam Sterilization Methods (Autoclave)

The principle of operation is that the boiling point of water increases with the increase of pressure in a humid environment, thus reaching temperatures above 100 °C. If the humidity is high in the environment, the sterilization efficiency of the temperature increases. Thermobile nutrient media, distilled water for rinsing, glassware, laboratory equipment are sterilized in the autoclave (Günaydın et al., 2015: Dolapçı, 2016).

Plant tissue culture media are generally sterilized at 121 °C and 1.05. kg / cm^2 (15.20 psi) in the autoclave. At this temperature autoclaving time, is affected by many factors, initially the volume of material to be sterilized and the way the autoclave was filled (Gürgün & Halkman, 1990).

The sterilization duration of the media in the autoclave varies according to the volume of the liquid (Table 2). Autoclave sterilization is a practical, and effective method. Autoclaving destroys fungi, viruses, yeast and bacteria. However, some chemical substances in the nutrient medium are not resistant to high temperatures and can break down (Ahmet et al., 2012).

In general, it should work with small volumes to prevent the degradation of substances in the nutrient solution with high temperatures. In addition, the container volume should be at least twice the nutrient volume in order to prevent boil over (Arditti, 2008). While this is not a problem in culture containers such as tubes, magenta, and glass jars, there is a problem with low-height containers such as petri dishes.

Table 2: Minimum autoclaving time in plant tissue culture depented to the volume of nutrient medium (Validation with aautoclave used is recommended) (Anonymous, 2020b)

Volume of medium for vessel (ml)	Application time (minute)
25	20
50	25
100	28
250	31
500	35
1000	40
2000	48
4000	63

Autoclaves are closed systems with an outer wall made of steel, resistant to inside pressure, and circulating steam inside. Autoclaves are costly but provide fast and effective sterilization. In addition, it is a method with a long sterilizing time, considering the heating, sterilization and cooling times.

Autoclave sterilization, consumes a lot of electricity. Besides, long sterilization times, high-temperature resistant containers are required for sterilization (Ahloowalia & Savingikar, 2004; Duan et al., 2019).

2.1.3. Sterilization Through Filtration

The cleaning of microorganisms or particles in a liquid or air by passing through a filter or membrane is called sterilization by filtration. The components degraded at high temperature is sterilized by filtration, in other words, liquids that cannot be sterilized in the autoclave is passed through a sterile filter. A small volume of liquids is suitable for filter sterilization. Disposable syringe filter (Wheel filter) with 0.2-0.22 µm pore size membranes usually made of cellulose acetate and cellulose nitrate is used for this process. Syringe filters are disposable. Pressure should be applied for the liquid to pass through the membrane, for this a syringe with a screw tip is used. Gentle positive pressure is applied to the tip of the injector for filtration. While the liquid less than 100 ml slowly passes through the filter, particles larger than 0.22 µm are held by the filter and sterilization takes place. The filtered sterile solution is mixed with the autoclaved sterile medium in laminar air flow chamber, which has cooled to 35-45 °C. In such applications, the quantities of ingredients in the nutrient medium must be calculated accurately (Gürgün & Halkman, 1990). Filtration is not a practical method if the liquid volume increases. It is a suitable method for sensitive experimental studies.

Filtration is used to sterilize the laboratory air as well as sterilization of liquids. For the filtration of the air, HEPA filters that can purify the airborne particles up to 0.3 microns from the air are used. HEPA filters are installed in ventilation systems of the laboratory, to sterilize the air coming from outside for changing the air of laboratory.

During inoculation, HEPA filters are used in laminar air flow chamber to create a sterile working environment. HEPA filters have a certain lifespan and should be replaced at the end of this period. Working in a clean, moisture-free inoculation room, and using pre-filter in the laminar air flow chamber increases the efficiency of the HEPA filter.

2.1.4. Sterilization with Microwave Energy (Irradiation)

Microwave energy is high-frequency radio waves of 2450 MHz. This group of waves is located in the region between Radio-TV waves and radar waves. Microwave rays make the water molecules vibrate at high speed, heat occurs as a result of friction, causing expansion of molecules including bacteria, viruses, and sterilization takes place. The volume of liquid media determines the application time. It is an effective and economical method in inactivating some viruses, bacteria, fungi (Sanborn et al., 1982).

Household-type microwave ovens are used for sterilization through microwave irradiation. The power of microwave ovens is important in the sterilization. The power of the oven varies between 300-1200 Watt (Tisserat et al., 1992). Shareef et al. (2019) reported that heating of the nutrient medium in different volumes for 3 minutes in a microwave oven provides a practical, easy, fast and energy-saving sterilization without any change in the quality of the nutrient medium.

The most important advantage of sterilization with microwave rays provides sterilization in a short time, with low investment and cost. However, it is not suitable for laboratories that prepare 30-100 liters of nutrient medium per day.

Life Technologies company is a leading manufacturer of plant tissue culture in India, recommends that the prepared nutrient medium be dispensed into small-volume containers in order not to be damaged by the temperature applied for a long time in microwave and autoclave sterilization. Minimum sterilization times in microwave oven with 1000 Watt power are given in the Table 3, depended to volume of nutrient medium. The duration of microwave sterilization also varies depending on the power wattage and the number of containers in the microwave oven. Validation with microwave used, is recommended (Anonymous, 2020b).

Table 3: Minimum sterilization times of nutrient medium in microwave oven (Anonymous, 2020b)

Volume of Medium Per Vessel (ml)	Min. Microwaving Time (min)
25	4-6
50	6-8
100	8-10
250	10-12

2.1.5. Sterilization Through Ultraviolet, Gamma, and X-Rays

These rays have different wavelengths. Ultraviolet rays (UV) are rays with a wavelength of 100-400 nm. and they destroy microorganisms in the air and surfaces that they can reach. UV rays are used for the disinfection of air, water, and smooth surfaces such as wrapping paper. Due to its inability to penetrate, absolute sterilization is not provided (Gürgün & Halkman, 1990). Ultraviolet rays damage the skin and eyes, cause decomposition in the nutrient medium, kill the plant material (Arditti, 2008). For this reason, it is generally used in the preparation and inoculation rooms of tissue culture laboratories, in the sterilization and disinfection of air and surfaces when not working in laminar air flow chamber.

Gamma and X rays are the rays with the highest frequency and the lowest wavelength among electromagnetic rays. It is used in the sterilization of disposable petri dishes and scalpel blades in tissue culture. The sterilization process is carried out by specialized persons and institutions (Dolapçı, 2016).

2.2. Chemical Sterilization

It is a sterilization-disinfection process using chemical substances. Generally, it is applied in liquid form, but also in small amounts as gas. Various chemical substances kill microorganisms with different modes of action. While the same chemical substance shows a sterilization effect at high concentrations, this effect is reduced at low concentrations. Usage areas of chemicals used for sterilization and disinfection vary depending on the factors such as microorganism density, desired sterilization power, properties of the material, ambient temperature, contact time etc. (Gürgün & Halkman, 1990).

Chemical substances used in chemical sterilization in tissue culture;

2.2.1. Alcohols

Ethyl alcohol and isopropyl alcohol are the most commonly used forms. Commercially, ethyl alcohol is available in 96% concentration, but 70% concentration is preferred over condensed form because the effectiveness of alcohol increases by adding water (dilution). This is because pure alcohol blocks proteins in the cell wall, causing the inability to penetrate into cell. Bech surfaces, hands, and plant material are disinfected with 70% alcohol. Isopropyl alcohol is used less often than ethyl alcohol (Gürgün & Halkman, 1990; Dolapçı, 2016).

2.2.2. Sodium Hypochlorite (NaOCl)

It is the most widely used, easily available, economical chemical in tissue culture. It is available in the market at a concentration of 5%, but generally, concentrations of 1% are used for the external (surface) sterilization of plant material. Besides the surface sterilization of plant material, sterilization of nutrient media is also done with NaOCl.

2.2.3. Calcium Hypochlorite ($Ca(ClO_2)_2$)

The destructive effect of sodium hypochlorite on tissues is the most important drawback. Calcium hypochlorite, on the other hand, does not damage tissues. Calcium hypochlorite is white in color, it is commercially available in powder form, it is extremely difficult to store and preserve due to its water absorption properties. Calcium hypochlorite solution is prepared by dissolving seven gram Ca hypochlorite in 100 ml water. After filtering the solution should be used within 12 hour (Arditti, 2008).

2.2.4. Mercuric Chloride ($HgCl_2$)

It is a toxic substance. Generally, concentrations of 0.01-0.1% are used in tissue culture for explant sterilization (Tiwari et al., 2012; Navya Swetha et al., 2017). Dissolves 7% in water and 33% in alcohol. It should be prepared in a fume cupboard, in a ventilated environment.

2.2.5. Hydrogen Peroxide (H_2O_2)

Hydrogen peroxide is a colorless liquid used as a biocide in sterilization and disinfection. Commercial products of hydrogen peroxide are in concentrations varying up to 3-5% active substance. It is generally used in explant sterilization and is also added to liquid nutrient media. Hydrogen peroxide shows a wide spectrum of activity against viruses, bacteria, yeast, and bacterial spores. Hydrogen peroxide is a environmentally friendly compound as it breaks down into water and oxygen (Vural & Çelen, 2005).

2.2.6. Peracetic Acid (CH_3CO_3H)

It denatures proteins and enzymes similar to hydrogen peroxide. It can inactivate bacteria, yeast, and fungi within 5 minutes at 100 ppm concentrations. It is generally used in the external sterilization of culture containers and plant material (Vural & Çelen, 2005). It is known to be used in the sterilization of nutrient medium (Cardosa & Imthurn, 2018).

2.2.7. Clorine Dioxide (ClO_2)

It is one of the chlorine-based chemicals. Chlorine dioxide acts by destroying the cell membrane and oxidizing the intracellular components of the microorganism (Vural & Çelen, 2005). It is used in the sterilization of nutrients and explants in tissue culture (Cardosa & Imthurn, 2018).

2.2.8. Sodium Dichloroisocyanurate (NaDCC; $C_3Cl_2N_3NaO_3$)

Sodium dichloroisocyanurate a chlorine compound is generally used as a water disinfectant in swimming pools. It is also added to nutrient media in tissue culture and is used in explant sterilization

2.2.9. Biosides

They are used to prevent fungal and bacterial contamination. It is added to the nutrient medium before or after sterilization in order to increase efficiency in sterilization and prevent contamination. It can be autoclaved. In addition, it is also added into rinsing water in explant sterilization. Plant preservative mixture (PPM) is the most well known biosides in tissue culture. Plant preservative mixture (PPM) is a mixture of two broad-spectrum industrial isothiazolone biocides, methylisothiazolone (MI) and chloromethylisothiazolone (CMI) (Nietz, 1998).

3. DIFFERENT APPLICATIONS IN STERILIZATION OF NUTRIENT MEDIUM IN ORNAMENTAL PLANT MICROPROPAGATION

3.1. Autoclave Sterilization of Nutrient Medium in Ornamental Plants

The use of autoclaves is the essential method in the sterilization of the nutrient medium. It is possible to give numerous research and commercial examples on sterilization of nutrient media with autoclave (Minerva & Kumar, 2013; Gök et al., 2016; Sharma, 2017; Özzambak et al., 2018; Oo et al., 2019). As mentioned before, the sterilization time with autoclave varies between 15 and 60 minutes depending on the volume of the nutrient medium. This period is the sterilization process to be applied after reaching 121 °C and 1.05kg / cm^2 pressure. Considering the warming period before sterilization and cooling period after sterilization, the total sterilization time varies between 2-4 hours.

After the nutrient medium is prepared and pH adjusted, agar is added if it is to be worked in a solid medium. After the agar is dissolved at 100 °C on a hot plate stirrer or microwave oven or water bath. Than nutrient medium is poured into the culture vessels as 15-50 ml and sterilized in an autoclave. Another application is to empty the nutrient medium into sterile culture containers in the sterile cabinet and leave to cool after autoclaving. In order to prevent the decomposition of the thermolabile materials that are damaged and decomposed at high temperatures in the autoclave, the second method should be used in case of filtration.

3.2. Autoclave Plus Filtration Method in *Portulaca grandiflora*

Filtration usually performs the sterilization of small volume liquids and solutions of thermolabile substances. Therefore, it is a complementary method of sterilization in the autoclave, not a single method. In the in vitro flowering study of *Portulaca grandiflora*, filter sterilized 0.5-2.0 mg / l GA_3 was added to autoclaved MS medium (Cruz et al., 2020). Similarly, in addition to GA_3, zeatin, 2-isopentyl adenine (2IP), zeatin, absisic acid (ABA), urea, some vitamins, pantothenic acid, antibodies, colchicines, plant extracts and enzymes thermolabile must be filter sterilized (Ahmed et al., 2012).

3.3. Microwave Oven Based Sterilization Of Media For Micropropagation Of *Phalaenopsis amabilis* And *Oncidium cebolleta*

This research aimed to simplify seedling cultivation by sterilizing the nutrient medium with a microwave oven without using autoclaves for small-scale orchid micro propagators and orchid enthusiasts. The nutrient medium was prepared in 2 liters, then the pH was adjusted to 5.6. The heated media was dispensed to eight 500 ml containers, vessels were placed on the microwave oven plate circularly. Five hundred mililiter of water was placed in the jar in the middle of the medium containers. During sterilization, the lids of the jars are kept loose to prevent overflow in the boiling phase and to prevent contamination by creating a vacuum during cooling.

After the media cooled, the lids are tightly closed. The efficiency of microwave oven sterilization was compared with autoclave (121 °C-15 min) sterilization. Nutrient media were microwave sterilized for 5, 8, 10, 15, 20 minutes. When the sterilization time with the microwave was over 15 minutes, no contamination occurred, however, the best plantlet growth was obtained from the medium where 2ml / l (30% H_2O_2) was added to the medium and heated for 8 minutes. They recommended the addition of 8 g / l agar for solidification, they used a microwave oven of 950 W and emphasized that 50ml of the medium would be the ideal volume in 200ml containers (Venturieri et al., 2013).

Life Technologies company, states that in addition to sterilizing the orchid nutrient medium with an autoclave (121 °C, 1.05kg / cm^2), it can also be successfully sterilized by using a microwave oven. (Anonymous, 2020b).

3.4. Chemical Sterilization of the Nutrient Medium in *Gerbera* Micropropagation

Gerbera is the cut flower where micropropagation is most commonly used commercially. Although gerbera seedlings are propagated by traditional vegetative methods (cutting), tissue culture micropropagation is preferred all over the world for a healthy, fast, and large number of seedlings (Minerva & Kumar, 2013; Navya Swetha et al., 2017; Rashmi et al.; 2018). Chemical sterilization of the nutrient medium instead of autoclave sterilization has been investigated to reduce labor and energy costs in this kind of intensive micropropagation.

3.4.1. Sterilization of the Nutrient Medium with Sodium Hypochlorite (NaOCl)

The study was carried out in the rooting phase of Gerbera micro propagation. Chemical sterilization of the nutrient medium was performed by adding 0.0005, 0.001, 0.002, and 0.003% active chlorine to the medium. 2% sodium hypochlorite bleach was used as an active chlorine source. Autoclaved nutrient medium, and the unsterilized medium without adding chlorine were considered as control treatments. In nutrient media sterilized with 0.0025 and 0.003% active chlorine, there was no contamination before and after inoculation, and there was no significant difference in in vitro plantlet growth compared to the autoclaved medium (Pais et al., 2016).

3.4.2. Sterilization of The Nutrient Medium with Chlorine Dioxide (ClO_2) and Paracetic Acid

Other chemicals used in chemical sterilization of the nutrient medium in gerbera were chlorine dioxide (ClO_2) and paracetic acid. The MS medium was sterilized with 0.0035, 0.070 and 0.0105% ClO_2 and 1.0-2.0-3.0 ml / l paracetic acid. Chlorine dioxide increased pH, paracetic acid decreased pH, and pH values were readjusted. Paracetic acid reduced the contamination rate on the nutrient medium, but contamination could not be eliminated completely. On the other hand, no contamination was detected in all chlorine dioxide concentrations. At the same time, no phytotoxicity was observed in *Gerbera* plantlets growing in media where ClO_2 was added (Cardoso & Imthurn, 2018).

Duan et al. (2019) sterilized culture dishes and nutrient media with chlorine dioxide in potato tissue culture and compared it with autoclaved media. They reported that there were no important difference between sterilization methods in terms of molecular level and micro tuber formation.

3.5. Sterilization of The Nutrient Medium with Essential Oils in *Chrysanthemum*

Plant extracts and essential oils are also used in the sterilization of the nutrient medium. Betel (Piper betle), cassumar ginger (*Zingiber cassumunar*), holy basil (*Ocimum sanctum*), clove (*Eugenia caryophylata*), lavender (*Lavandula angustifolia*), lemon (*Citrus lemon*), bergamot (*Citrus bergamia*), tumeric (*Curcuma longa*) have been used in the sterilization of the nutrient medium in the propagation of various plants.

Thepsithar et al. (2013) distributed the MS medium into 120 ml glass containers at 25 ml volume and added betel oil, bergamot oil, cinnamon oil, lavender oil to each container as 18-324 µL. for in vitro chrysanthemum culture. The prepared medium was kept at room temperature for two weeks before explant inoculation to detect contamination, and 5-7 mm in vitro chrysanthemum shoots were planted in media without contamination. They reported that full sterility was not achieved in any essential oil at a dose of 18 µL. On the other hand, 36 µL for betel and cinnamon oil, 108 µL for lavender and bergamot oil, 324 µL for tumeric oil doses provided 100% sterility after inoculation.

However, *Chrysanthemum* shoots grew only in MS medium containing 36 µL cinnamon oil, while no growth was observed in media containing other essential oils. Besides, cinnamon and lavender oil (1/3) were mixed with 10% povidone-iodine and 2% iodine + 2.4% KI and added to the medium at a dose of 36-72 µL. In these media, researchers detected shoot growth less than the medium sterilized in the autoclave. Some essential oils are effective in sterilization, but they must be tested for plant growth.

3.6. Sterilization of The Nutrient Medium with NaOCl in *Ananas comosus* Tissue Culture

Ananas comosus is a succulent ornamental plant in Mediterranean climate and a fruit plant in the tropical region. Its propagation is done by tissue culture. In the study conducted by Teixera et al. (2006) with this plant, the nutrient medium was sterilized by autoclave and NaOCl, and media were compared with each other. After the nutrient medium (MS) was prepared, NaOCl was added, after 15 minutes, the pH was adjusted. Treatments are arranged as 0.0001, 0.0003, 0.0005, 0.0007, 0.0009% active chlorine. No contamination was observed starting from 0.0003% concentration, and they obtained values twice the autoclave media in terms of average shoot number/culture and fresh weight. They state that the nutrient medium can be sterilized with 0.0003% active chlorine without causing any harm to the culture.

Other chemical sterilizers tested in the sterilization of the nutrient medium other than NaOCl are, $HgCl_2$, $AgNO_3$, fungicides (carbendazim, propiconazole, hexaconazole, tebuconazole), plant extracts (*Morinda citrifolia, Azodirachta indica leaf, Allium sativum,* and *Allium cepa*) (Flores et al., 2012). Among these, it is reported by Adilackshmi et al. (2015) that the alternative to autoclaving is NaOCl application, as a result of the study conducted on sugar cane.

3.7. Sterilization of The Nutrient Medium with NaOCl in *Eucalyptus* Tissue Culture

Eucalyptus benthamii is an endemic tree originating from Australia. Superior genotypes are clonally propagated by mini cuttings and micro cuttings. The possibility of sterilization of the nutrient medium with NaOCl was investigated in order to reduce the high cost of micropropagation and the need for technical equipment. Culture containers (0.001%) were washed with NaOCl water and nutrient media were chemically sterilized with the addition of 0.001%, 0.003, 0.005, 0.007, 0.010 (v / v) active chlorine.

The experiment was conducted in the establishment stage. At the end of the trial, it was reported that survival rates of the explants at the end of the first 28 days, decreased compared to sterilization through an autoclave, with the increase of CI concentration. Brondani et al. (2013) stated that there was no statistical difference between the media in terms of fungal and bacterial contamination, and considering the number of shoots and length of shoot per explant, 0.001% and 0.003% active chlorine concentration was sufficient for the sterilization of the nutrient medium.

3.8. Micropropagation of *Cymbidium, Phalaenopsis, Carnation, Chrysanthemum* Using Chlorine-based Disinfectants in Different Stages

Yanagawa et al. (2006) used four types of chlorine disinfectants in order to realize simpler micropropagation that will reduce dependence on high-cost devices. They sterilized the nutrient medium with sodium hypochlorite, sodium dichloroisocyanurate, chlorine bleaching agent, and calcium hypochlorite, and performed explant planting on nutrient medium without using laminar flow. Also, sterile explants were planted in a nutrient medium under non-sterile conditions, sprayed chlorine solutions with 0.005-0.001% active chlorine to ensure sterility, and compared with cultures that were sterilized in the autoclave and performed inoculation in laminar flow.

Researchers found that all chlorine disinfectants were effective in the sterilization of nutrient media. Furthermore, they reported that spraying the nutrient medium and the explant with a disinfectant in non-sterile conditions were sufficient to sterilize the explant and the nutrient medium. They explained that these techniques could be used in the culture of chrysanthemum and cymbidium shoot tip, stem explants in chrysanthemum and carnation, cymbidium PLB explants, and cymbidium and phalaenopsis plantlets without contamination and were not cause any damage to the tissues.

3.9. Using Different Sterilizing Agents Alternative To The Autoclave in *Phalaenopsis* Protocorm Growth

Phalaenopsis protocorm grown on cost effective medium (Hyponex medium) and some chemicals that could be alternatives to autoclaving were investigated. Sterilizing agents, 10% povidone-iodine, 5.25% sodium hypochlorite, 2% merbromin solution or 0.1% acriflavin solution, were added in a 120 ml glass jar containing 25ml of heated liquid or solid culture medium, in various concentrations (30 – 450 µL) alone or in combinations with 2.5% iodine + 2.5% potassium iodide. For comparing, all media were kept in room temperature for 2 weeks to investigate effects of sterilizing agents on sterile conditions of media. Protocorms of Phalaenopsis were cultured for 14 weeks on both media. Researchers found that 90µL of 10% povidone-iodine, 150 µL of 5.25%

sodium hypochlorite, 150 µL of 2% mercurochrome, 90 µL of 2.5% iodine + 2.5% potassium iodine in combination with 10% providone-iodine (1:3) and 30µL of 2.5% iodine + 2.5% potassium iodide in combination with 2% mercurochrome showed 100% sterile conditions in liquid medium but provided 75, 100, 50, 75 and 80% sterile conditions, respectively, in solid medium. For growth of Phalaenopsis protocorms, 90 µL of 10% povidone-iodine in liquid Hyponex medium gave the comparable growth of protocorms to control medium while 150µL of 5.25% sodium hypochlorite in solid medium provided the promising growth of protocorms (Thepsithar & Thongpukdee, 2013).

CONCLUSION

Ornamental plants are the plant group where micropropagation is applied the most (Özzambak, 2010). The reason for this is that vegetative methods are generally used for the propagation of ornamental plants. The most important problems in micropropagation, where fast and intensive production takes place, are the high cost due to reasons such as low multiplication rate, contamination, intensive labor and energy costs, equipment and tool requirement, usage, and expenses.

One of the attempts to reduce the high cost is the research and use of alternative methods for sterilization of the nutrient medium instead of the conventional sterilization method with autoclave. Autoclaving is a process that consumes a lot of energy, is long-term and affects some substances in the nutrient medium. In the article, the methods used instead of autoclaving are explained and examples from the researches and results conducted on this subject are given.

As a result of both the research results and the studies we have done on this subject, the method that can replace sterilization with autoclaving can be stated as sterilization of the nutrient medium with NaOCl. This can be attributed to the fact that NaOCl destroys bacteria, fungi, and viruses by reactivating amino acids, nucleic acids, amines, due to its strong oxidizing property (Yıldız et al., 2012).

However, the important subject here is that besides the sterilization of the nutrient medium, explant development, in vitro shoot formation, and propagation rate are not affected negatively. Therefore, it is necessary to conduct different studies, researches to determine the most appropriate NaOCl concentration. Chemical sterilization will also be useful in preventing contamination, which is a major problem in bioreactor culture (Etienne & Berthouly, 2002).

Sterilization of the nutrient medium with microwave energy is another alternative method to autoclave sterilization (Youssef, et al., 2001; Vora & Jasrai, 2012). In traditional heating methods, heat is transmitted from the surface of the substance to the interior. When compared to these methods, during microwave heating, the heating rate is high and the processing time is short as heat is generated inside the material. It has been stated that vitamin and mineral losses are less in microwave-processing. Other advantages are that the equipment takes up less space, is easy to clean and saves energy during the process. Not using the autoclave in tissue culture is important in terms of saving energy costs as well as saving money for an expensive device as an autoclave (Savangikar, 2004). In this respect, it attracts the attention of small entrepreneurs and those who make tissue culture as a hobby and who are looking for low-cost production options. However, overflow during boiling, excessive evaporation, and volume changes are important bottlenecks for sensitive studies. In addition, as the volume of the medium to be sterilized increases, sterilization with microwave becomes difficult and sometimes, contamination problems may arise. For this reason, some biocides or H_2O_2 are added to the nutrient medium during sterilization with microwave energy (Venturieri et al., 2013). Standard sterilization cycles at humid heat (autoclaving processes) are operated at 121 °C and 1.05 kg/cm^2 pressure. It is argued that if larger volumes of microwave apparatuses can be built that allow similar pressure to develop, it could be an interesting option for routine sterilization of the laboratory supplies and large volume of nutrient media (Trivedi et al., 2011).

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CHAPTER 17

PROPAGATION OF TERRESTRIAL ORCHIDS: CURRENT APPROACH AND FUTURE DIRECTIONS

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INTRODUCTION

Since ancient times, flowering plants have found their application in horticulture, the pharmaceutical, cosmetic, and food industries. The most numerous flowering family belonging to this group of plants is the family Orchidaceae Juss, which, with over 850 genera and about 25,000 species, makes up about 10% of the total number of flowering plants (Chase et al., 2003; Roberts & Dixon, 2008).

The species belonging to the Orchidaceae family differ according to the substrate they inhabit and, as such, can be divided into four groups: epiphytes, terrestrial species, lithophytes, and saprophytes. Epiphytes are the most numerous, accounting for 70% (Gravandeel et al., 2004), while terrestrial orchids account for about 25% (Hossain et al., 2013). The Orchidaceae family is also characterized by a high degree of distribution, making it a cosmopolitan family. With a quarter of all known species, Northern Andes and Ecuador represent the richest area in epiphyte orchids (Cribb et al., 2003; Pérez-Escobar et al., 2017). Sumatra follows with over 1200 species (Comber, 2001), Madagascar with almost 1000 species (Hermans et al., 1999), and Borneo with over 720 species, where only epiphytes occur (Wood et al., 1993). The Indochina Peninsula also represents an orchid-rich (epiphytic and terrestrial) region, while the southwest of Australia is known as the richest center for terrestrial orchids (Cribb et al., 2003). The temperate zone is another natural habitat for orchids, but in contrast to the tropics, there is a much smaller number of species. According to Gladkova (1982), 170 species grow in North America, 215 species from 36 genera grow throughout Europe (Stewart, 1989), and while Dumont et al. (1996) state that over 300 species of terrestrial orchids have been registered in Europe, North America and the Middle East. These indications suggest that the temperate zone is a natural habitat for a much smaller number of orchid species than the tropical zone. Terrestrial orchids, commonly known as ground orchids, include about 4000 species (Dressler, 1981).

In this chapter, we will briefly explain the reproduction mechanism of terrestrial orchids and give an overview of the *in vitro* techniques used for their propagation. We will discuss factors that impede the survival of natural populations of terrestrial orchids and the use of *in vitro* techniques to combat these threats. We will also discuss the influence of *in vitro* techniques on the commercialization of terrestrial orchids as ornamentals.

1. REPRODUCTION OF TERRESTRIAL ORCHIDS

The life cycle of orchids depends on their interaction with other organisms. They have developed a pollination system with various deceptive strategies, such as general food deception, food deceptive flower imitation, imitation of the hatchery, imitation of the shelter, pseudoantagonism, rendezvous attraction, and sexual deception known as pseudocopulation (Tremblay et al., 2005; Jersakova et al., 2006). After pollination and successful fertilization, the capsule formed can contain between 1,300 and 4,000,000 seeds (Arditti, 1967).

1.1. Seed Characteristics

Orchids are characterized by tiny seeds (Figure 1) that are 0.25 to 1.2 mm long (Knudson, 1922; Rasmussen, 1985) and weigh 0.3 to 14 μg (Harley, 1950). Their seed structure and size make them suitable for wind distribution (Rasmussen, 1995). However, seed germination of terrestrial orchids is a complex process, so a large number of the seeds and their easy distribution do not necessarily promise reliable germination that would ensure an expansion of the existing population or the formation of a new one. The tiny seeds of orchids cannot germinate by themselves, and various biotic and abiotic factors determine germination, which is a limiting step in developing a new plant. Their reproductive ecology, a complicated and slow cessation of seed dormancy under *ex situ* conditions, characterize orchids growing in temperate climates (Butcher & Marlow, 1989). Seeds of terrestrial orchids have a particular form of morpho-physiological dormancy, which is composed of morphological (presence of an undifferentiated embryo and a strong seed coat) and physiological determinants (the embryo does not have sufficient growth potential to penetrate the seed coat and germinate) (Baskin & Baskin, 2014). Moreover, the seeds do not have an endosperm, and the nutrient content of mature seeds is concentrated in the embryonic cells. The nutrients in seeds include lipids, proteins, and carbohydrates, and their quantity varies from species to species (Rasmussen, 1995). Although embryos contain carbohydrates (sucrose, glucose, fructose, maltose, rhamnose), the amount of sugar present is usually insufficient to support germination to the end or even to be used before germination begins (Manning &

Van Staden, 1987). Embryos also lack enzymes that convert lipids into free sugars and cannot metabolize polysaccharides and lipids (Manning & Van Staden, 1987). Such a specific seed structure makes the biology of seed germination unique. It is primarily based on the formation of protocorm, the non-photosynthetic developmental stage between seed germination and the production of the first leaf (Baskin & Baskin, 2014). Due to the lack of nutrients, the onset of germination and the protocorm formation is caused by the formation of symbiosis with the corresponding mycorrhizal fungi, which supply the embryo with water, carbohydrates, minerals, and vitamins (Rasmussen, 1995; Kauth et al., 2008).

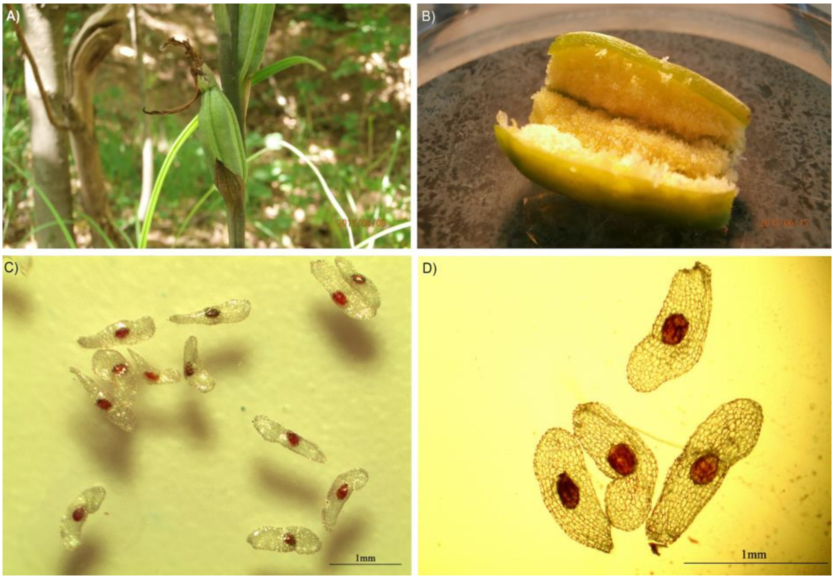

Figure 1: *Limodorum abortivum*, a-b) green capsule with seeds; c-d) viable seeds (Original by Ostojić)

1.2. Orchid Mycorrhiza

The orchid mycorrhiza has a significant influence on the life cycle of orchids. Mycorrhizal fungi (Figure 2) provide seeds with carbon that promotes seed germination and seedling formation, and this relationship is maintained throughout the life cycle of many species (Abadie et al., 2006; Rasmussen & Rasmussen, 2007). The orchid mycorrhiza belongs to the endomycorrhiza group, whose main characteristic is that the fungal mycelium inhabits the intracellular spaces of the cells and parenchymal tissue of the root or seed, after which it penetrates the cortex cell and forms a peloton (Figure 2C). This type of mycorrhiza is unique in that it occurs exclusively within Orchidaceae. Reisek first discovered this type of mycorrhiza in 1847 (Hadley, 1982), and the development of a protocol for obtaining a pure culture of endophytes was continued in further decades (Ramsay et al., 1986). The taxonomy of fungi and the physiology of association have mainly been the subject of earlier studies, and in recent years research has been complemented by molecular analysis. (Rasmussen, 1995; Sharon et al., 2008; Kohler et al., 2015). Fungi that live in symbiosis with orchids belong in most cases to the genus *Rhizoctonia* (*Rhizoctonia* - like fungi) and can be in the anamorphic or teleomorphic stage.

Conventional methods for the identification of fungi of the genus *Rhizoctonia* were based on the morphological characteristics of the culture, which included colony color, the formation of exudates and sclerotia, and the structural features of the hyphae, i.e., the presence of monilioid cells and the number of nuclei in young cells (Currah et al., 1997; Snech et al., 1991). The genus *Rhizoctonia* comprises polynucleate, binucleate, and multinucleate strains according to the number of nuclei in the cells. The most common plant pathogen *Rhizoctonia solani* (Teleomorphs: Thanatephorus, Ceratobasidiales) is distinguished as a multinucleate type. Fungi of the genera *Ceratobasidium* (Ceratobasidiales) and *Tulasnella* (Tulasnellales) possess binucleate cells, while mononucleate strains are found in anamorphs of *Ceratobasidium* (Hietela et al., 2001).

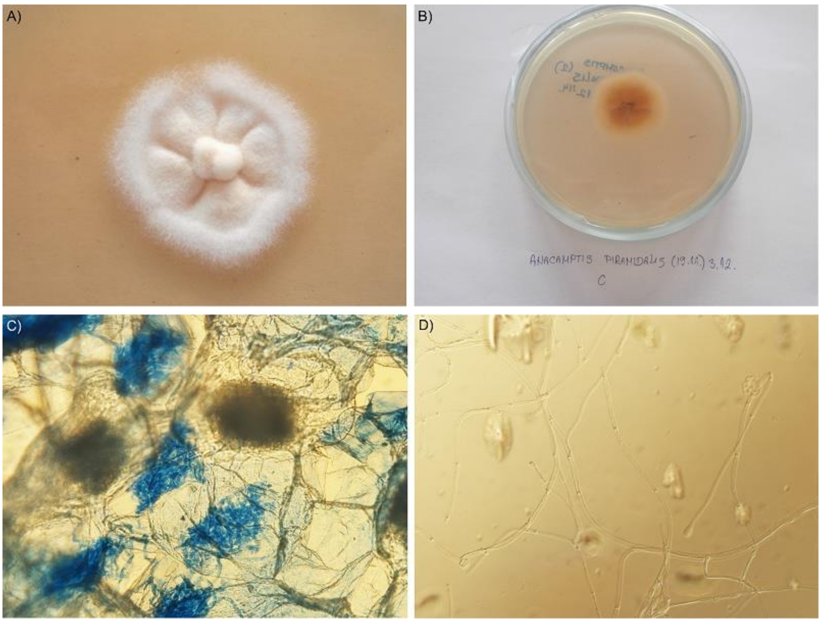

Figure 2: Fungi isolated from the root of *Anacamptis pyramidalis*. a) aerial mycelium, b) substrate mycelium, c) pelotons in root cells (stained blue), d) fungal hyphae (Original by Ostojić)

Orchid mycorrhiza can also be established with saprophytic, non-mycorrhizal fungi (*Cylindrocarpon*, *Fusarium*, *Trichoderma*) (Snech et al., 1991; Currah et al., 1997; Gezgin & Eltem, 2009). Furthermore, there is a difference between the symbiosis established with the autotrophic and the mycoheterotrophic host. Autotrophic terrestrial orchids form mycorrhiza with anamorphic genera such as *Ceratorhiza*, *Epulorhiza,* and *Moniliopsis* (Currah et al., 1997). Most of these symbionts produce enzymes such as cellulase and polyphenol oxidase, which enable the organic matter in the soil to break down into simple sugars. In contrast to them, mycoheterotrophic and mixotrophic orchid species live in symbiosis with ectomycorrhizal fungi such as Thelephoraceae and Russulaceae (Abadie et al., 2006), through which they connect with the host trees or shrubs and are thus supplied with photosynthetic products of the autotrophic host. The teleomorphic form of *Rhizoctonia*-like fungi includes three basidiomycetes - Tulansellaceae, Sebacinaceae, and Ceratobasidaceae (Peterson et al., 1998; Sharon et al., 2008), while fungi of the genera *Elorhiza*, *Moniliopsis,* and *Rhizoctonia* have an anamorphic stage (Moore, 1988).

The genus *Rhizoctonia* represents the largest group of binucleate fungi living in symbiosis with orchids. Their primary identification was based on morphological features such as the number of nuclei per hyphae cell and anastomosis group. The endophytic fungus most commonly living in symbiosis with terrestrial orchids is *Tulasnella calospora*. This fungus has been isolated from the roots of *Dactylorhiza purpurella* and *Anacamptis laxiflora* as well as from the roots of many terrestrial and epiphytic orchids growing on the Australian continent. It is widely distributed throughout the world and can be found in every ecosystem, from tropical to temperate climates. Furthermore, strains belonging to this species are compatible with several temperate orchid species (Rasmussen, 1995; Kohler et al., 2015). *Ceratobasidium cornigerum* is another endophytic fungal species that lives in symbiosis with terrestrial orchids. It has been isolated from the roots of *Goodyera repens* and *Dactylorhiza purpurella* and strains of this species successfully establish mycorrhiza *in vitro* with temperate terrestrial orchids (Rasmussen, 1995).

2. *IN VITRO* TECHNIQUES IN THE PROPAGATION OF TERRESTRIAL ORCHIDS

The germination of seeds is a crucial event in the life of a plant and depends on many direct or indirect factors, especially in the case of terrestrial orchids. For terrestrial orchids, mycorrhizae are obligatory to support mycoheterotrophic embryos and protocorms, as they obtain energy, carbon, and mineral nutrients from the fungi (Rasmussen, 1995; McCormick et al., 2009). This mycoheterotrophic stage can last several months or even years under natural conditions. For a successful symbiosis, germination of the seeds, and further growth of the seedlings, suitable growth conditions such as temperature, light, humidity, and oxygen are also required. These elementary conditions make the germination of the seeds of terrestrial orchids a complicated and lengthy process. In recent years, however, germination under controlled *in vitro* conditions, which implies both symbiotic and asymbiotic seed germination, has proven to be the most efficient method for rapid multiplication (Johnson et al., 2007).

2.1. Symbiotic Seed Germination

The *in vitro* symbiotic seed germination represents a procedure with the established fungus/seed symbiosis under sterile and controlled conditions (Zettler, 1997). A good side of this procedure is that we can directly follow the development of the symbiosis and investigate the specificity of the isolated mycorrhizal fungi (Stewart & Kane, 2007). On the other hand, it is a very long and relatively expensive process. It is difficult to establish a symbiosis *in vitro*, as incompatibilities often occur between isolated fungi from the root of the mother plant and seeds of the same species. This means that symbiosis with the isolated fungus cannot be successfully established, so germination cannot be initiated (Pierce et al., 2010). For example, the fungus *Rhizoctonia solani* isolated from the root of *Dactylorhiza umbrosa* did not promote seed germination and the formation of protocorm in *Dactylorhiza iberica*, *Dactylorhiza umbrosa,* and *Orchis palustris* (Çığ & Yılmaz, 2017). Furthermore, the relationship between orchid species and fungal isolates can vary from symbiosis to parasitism (Ovando et al., 2005). Isolates of *R. solani* express the pathogenic effect on the seeds of *Cattleya skinneri*, although they live in symbiosis with adult individuals. In contrast, the pathogenic effect of *R. solani* isolates on seeds of *Bipinnula fimbriata* has not been registered, but neither has rhizoid formation been promoted by this fungus (Steinfort et al., 2010). The correct choice of fungus intended for symbiotic seed germination influences both intensity and germination rate. While a symbiosis was established between the orchid *Habenaria macroceratitis* and the fungal isolate of the anamorphic genus *Ceratorhiza*, resulting in 63.7% germination after 83 days (Stewart & Zettler, 2002), the symbiosis with the fungal isolate of the anamorphic genus *Epulorhiza* resulted in a higher germination rate (65.7%) and a shorter germination period (58 days) (Stewart & Kane, 2006a). The mentioned difficulties in finding the compatible fungal partner for the symbiosis can be avoided with the asymbiotic technique, where a compatible nutritive medium replaces the fungal partner. Although successful symbiotic seed germination initially established a higher degree of germination and protocorm formation than the asymbiotic protocol (Qien et al., 2008), recent advances in the asymbiotic technique offer an equally good germination percentage (Bustam et al., 2014).

2.2. Asymbiotic Seed Germination

Asymbiotic *in vitro* seed germination is a unique technology to study the effects of various abiotic factors on seed germination and seedling development without the mediation of fungi. This *in vitro* technique was introduced in 1922 by the American botanist Lewis Knudson who discovered that salep contains 48% mucilage, 27% starch, 5% protein, and only a few percent sugar and soluble minerals. Based on this knowledge and some earlier findings that mycorrhizal fungi can cause the decomposition of sugar from sugar cane (Bernard, 1909), it was concluded that fungi stimulate seed germination by decomposing starch, pentose, and nitrogen compounds and most likely provide the seeds with growth-stimulating compounds. This assumption was confirmed by Knudson (1922, 1924), who established the asymbiotic seed germination of species of the genera *Cattleya*, *Laelia,* and *Epidendrum* on a medium containing sugar and minerals (Arditti, 1967). His research represents a milestone in the germination of orchid seeds under controlled conditions, after which this protocol has been further improved through intensive research (Van Waes & Debergh, 1986; Kitsaki et al., 2004; Kauth et al., 2006; Pierce et al., 2013). Asymbiotic seed germination has been successfully established in the following species of terrestrial orchids: *Cypripedium debile* (Hsu & Lee, 2012), *Cypripedium macranthos* (Huh et al., 2016), *Cypripedium lentiginosum* (Jiang et al., 2017), *Dactylorhiza osmanica* (Sazak & Ozdener, 2006), *Anacamptis palustris* (Magrini et al., 2011), *Cephalanthera falcata* (Yamazaki et al., 2006), *Bletia purpurea* (Dutra et al., 2008; Johnson et al., 2011), *Orchis coriophora* (Bektas et al., 2013) *Orchis mascula* (Valletta et al., 2008) *Himantoglossum jankae* (Dulić et al., 2019), *Spiranthes spiralis* (Dulić et al., 2019) as well as few species from the genus *Ophrys* (Kitsaki et al., 2004; Dulić et al., 2018).

The biology of seed germination is the same for all species and includes two critical developmental phases - embryo swelling and the formation of the protocorm (Figure 3). However, numerous factors such as seed maturity, interruption of dormancy and seed sterilization, composition, and content of the nutritive medium, illumination, and temperature have a strong influence on seed germination (Arditti, 1967; Rasmussen, 1995; Kauth et al., 2008; Zeng et al., 2014, Dulić et al., 2019). It is, therefore, necessary to establish a protocol for each species so that the germination, growth, and development of plants can be faster and more productive.

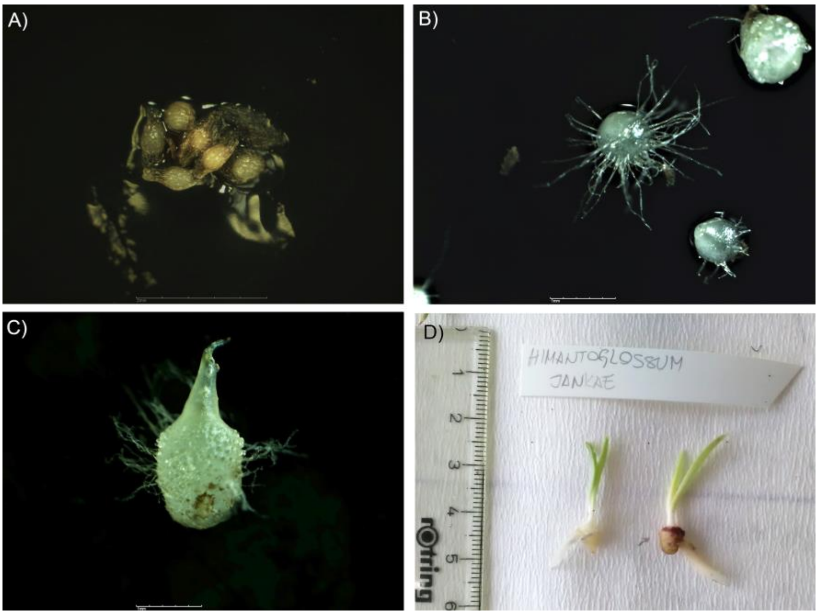

Figure 3: Asymbiotic germination of *Himantoglossum jankae* seeds, a) embryo with seed coat, b) formation of rhizoids, c) further development - formation of the shoot, d) well-developed bipolar seedling (Original by Ostojić)

The technique of asymbiotic seed germination can include both immature and mature seeds, and both methods have their advantages and disadvantages. Recently, germination of immature seeds has been increasingly used because it prevents seed dormancy and, according to some authors, reduces the possibility of infection (Lauzer et al., 1994; Light & MacConaill, 1998). However, the use of immature seeds implies the identification of the optimal stage of capsule development, which requires continuous monitoring of plant phenology (Kitsaki et al., 2004; Jiang et al., 2017). Furthermore, the technique of immature seeds requires rapid introduction into the culture, and the seeds should not be subjected to drying treatments. Therefore, there is no possibility of prolonged storage, while mature and well-dried seeds can be stored for longer periods (Seaton et al., 2010). Although there are certain obstacles to using mature seeds for asymbiotic germination, such as seed dormancy or higher infection rates, these problems can be easily overcome by protocols. The application of appropriate chemical treatments leads to successful seed sterilization and seed coat breaking (Van Waes & Debergh, 1986; Rasmussen, 1995; Valletta et al., 2008). The embryo becomes capable of absorbing water, which leads to the end of physiological dormancy (Rasmussen, 1995; Baskin and Baskin, 2014). Sodium (NaOCl) and calcium hypochlorite ($Ca(ClO)_2$) solutions have been successfully used to decontaminate and interrupt physiological dormancy (Ervin & Wetzel, 2002). Still, inappropriate concentration or duration of treatments may damage the embryos and impair germination capacity (Mweetwa et al., 2008). Calcium hypochlorite significantly stimulated the formation of protocorm in *Dactylorhiza incarnata* subsp. *serotina*, *Dactylorhiza maculata* subsp. maculata, *Liparis loeselii*, while sodium hypochlorite significantly inhibited germination in these species (Vejsadova, 2006). On the other hand, 1% NaOCl stimulated seed germination and protocorm formation in *Oreorchis coreana* Finet, while prolonged treatment with the same compound reduced germination (Bae et al., 2013).

The content of minerals and their available forms in the nutritive media represents the essential part of plant tissue culture. Numerous studies have shown that the asymbiotic germination of terrestrial orchid seeds is mainly due to the presence of nitrogen and nitrogen compounds in the nutritive media (Rasmussen, 1995; Kauth et al., 2006; Stewart & Kane, 2007; Sgarbi et al., 2009; Ponert et al., 2013; Dulić et al., 2019). The Knudson C nutritive medium, which contains ammonia and nitrogen in nitrate form, positively affects the germination of seeds of *Encyclia boothiana* var. *erythronolide* and *Calopogon* tuberosus (Stenberg & Kane, 1998; Kauth et al., 2006). The species *Bletia purpurea*, which inhabits North America, also successfully germinates on substrates with organic and inorganic nitrogen forms, indicating the ability of this species to adapt to a broader range of nitrogen forms (Dutra et al., 2008). In contrast, inorganic nitrogen forms had an inhibitory effect on the germination of *Limodorum abortivum* (Sgarbi et al., 2009). Similarly, for the species *Himantoglossum jankae*, *Ophrys sphegodes,* and species of the genus *Limodorum* higher germination rates were observed on a medium with organic nitrogen forms than on a medium with inorganic nitrogen forms (Magrini & De Vitis, 2017; Dulić et al., 2018; Dulić et al., 2019).

For most terrestrial orchids, organic additives, which are a source of amino acids in the nutritive media, have a strong influence on the germination of the seeds. Coconut water, banana powder, peptone, casein hydrolysate, yeast extract, and pineapple juice are the most commonly used organic additives in *in vitro* culture. However, these additives are not suitable for all species, do not always trigger germination when added to the nutritive medium, and may have an inhibitory effect on some species (Chand, 1991; Roy & Banerjee, 2002; Gupta, 2016). It is therefore essential to study the influence of organic additives on germination and to adapt them to each orchid species.

Coconut water is a liquid endosperm isolated from the coconut fruits and contains the hormone zeatin and less represented kinetin as well as other substances such as sugar, a variety of amino acids and vitamins that stimulate the germination and further growth of orchids (Gupta, 2016). The positive effect of this supplement on germination was found in the species *Cypripedium macranthos*, *Eulophia nuda*, *Spiranthes spiralis*, *Himantoglossum jankae,* and many species of the genus *Ophrys*, where a significantly higher percentage of germination was found on the medium enriched with this supplement (Kitsaki et al., 2004; Nanekar et al., 2014; Huh et al., 2016; Dulić et al., 2019).

Casein hydrolysate is a complex of amino acids that is often an integral part of the nutritive medium used to germinate seeds of terrestrial orchids (Gupta, 2016). It positively affects seed germination of an endangered medicinal orchid *Eulophia nuda* (Nanekar et al., 2014). The species *Rhynchostylis retusa*, *Cymbidium elegans, Cypripedium calceolus,* and *Epipactis helleborine* also showed a significantly higher germination rate and a shorter time span for germination on a casein-enriched nutritive medium (Van Waes & Debergh, 1986; Kumar et al., 2002). On the other hand, the presence of casein did not trigger seed germination in *Listera ovata* and *Dactylorhiza maculata* (Van Waes &

Debergh, 1986), while the inhibitory effect of this additive was found in the species *Coelogyne barbata* and *Herminium lanceum* (Chand, 1991).

Peptone is a water-soluble protein hydrolysate containing high concentrations of amino acids, and its effect on germination varies from species to species. It has a stimulating effect on seed germination of *Epidendrum ibaguense* (Hossain, 2008), *Calopogon tuberosus* (Kauth et al., 2008), *Eulophia cullenii* (Decruse et al., 2013), *Dactylorhiza maculata* subsp. *masculata*, *Dactylorhiza incarnata* subsp. *serotina* and *Liparis loeselii* (Vejsadova, 2006). In contrast to this species, an inhibitory effect was found in *Vanda tessellata* (Roy & Banerjee, 2002), whereas no positive or negative effect was observed in *Calanthe discolor* (Kano, 1965).

Illumination is one of the most important environmental factors in the germination of seeds of terrestrial orchids, which has not yet been sufficiently studied. However, because of its importance and direct influence on germination, several studies have investigated the impact of this factor *in vitro*, including the individual influence of illumination, the effects of illumination in combination with different nutritive media or temperature, the quality and quantity of light, and the influence of illumination on different phases of seed germination and seedling development (Fukai et al., 1997; McKinley & Camper, 1997; Kauth, 2005; Kauth et al., 2006; Dutra et al., 2008; Stewart & Kane, 2006b; Godo et al., 2010; Godo et al., 2011; Johnson et al., 2011, Johnson & Kane, 2012; Chen et al., 2015; Quiroz et al., 2017; Godo et al., 2011).

In most species, light has an inhibitory effect on the germination of seeds of terrestrial orchids. However, there are species in which the seeds germinate under illumination, and the *in vitro* germination responses to photoperiods are often species-specific (Arditti et al., 1981; Kauth et al., 2008). For example, seeds of *Himantoglossum jankae* germinate only in the dark, while seeds of the species *Spiranthes spiralis* can germinate both in the darkness and in 16-hour photoperiods (Dulić et al., 2019). The taxonomic affiliation and differences in the species' environment are reflected in the light requirement of the seed during germination (Rasmussen, 1995). Terrestrial orchids native to Australia have the highest germination rate under 16-hour photoperiods. In contrast, even the weakest illumination can have an inhibitory effect on germination in species from the Holarctic region (Van Waes & Debergh, 1986; Yamazaki & Kazumitsu, 2006). However, illumination can have various effects on seed germination in species from temperate climate zones (Arditti et al., 1981). The species *Bletia purpurea*, which inhabits North America, shows a significantly higher germination rate under 16-hour illumination (Dutra et al., 2008). A similar preference was observed in *O. mascula*, where seed germination did not occur in complete darkness (Valletta et al., 2008). Also, in *Calopogon tuberosus* (Kauth et al., 2006) and *Orchis coriophora* (Bektas et al., 2013), a significantly faster seedling development under 16-hour illumination was achieved. In contrast to these species, which require special light conditions, the seeds of *Goodyera oblongifolia* and *Spiranthes spiralis* showed a high germination rate both in complete darkness and under light exposure

(Arditti et al., 1981; Dulić et al., 2019). Although the influence of light on seed germination has been the subject of many studies, it is still necessary to determine the optimal conditions for seed germination and seedling development for each orchid species.

3. FUTURE DIRECTIONS AND RESEARCH PRIORITIES

3.1. Challenges to Sustaining Natural Populations of Terrestrial Orchids

Terrestrial orchids are of great importance for horticulture and landscape architecture because of their overly ornamental value, their long life and flowering period, and their ability to grow under temperate conditions. However, in contrast to commercial orchids (e.g., *Phalaenopsis* spp.), the best-selling flowering plants in Europe, terrestrial orchids are still difficult to find on the horticultural market. The main reasons for the low penetration of terrestrial orchids in the markets are explained by Kauth et al. (2006) with the limited production within amateur producers, consumer ignorance about this group of orchids leading to low demand and interest from the industry, and a complicated process for generative propagation.

However, some terrestrial orchids have found significant use in the food, pharmaceutical, and medical industries. This type of use goes back to ancient times, especially to Chinese and Japanese culture. In Europe, the ancient Greeks and Romans were the first consumers of orchids (Bulpitt, 2005). Today, the best-known product from the underground organs of orchids, the so-called salep, has a wide application in the production of ice cream, sweets, and hot drinks (Tamer et al., 2006). The largest salep producers are Turkey, which produces between 15 and 20 tons of salep per year, followed by Greece, Iraq, and Albania (Ghorbani et al., 2014; Quave & Pieroni, 2015; Kreziou et al., 2016). The species most commonly used in the food industry belong to the genera *Anacamptis* Rich, *Dactylorhiza* Neck, *Himantoglossum* Spreng, *Oprhrys* L., *Orchis* L., *Separis* L., and *Steveniella* Schltr (Figure 4) (Kreziou et al., 2016; Ghorbani et al., 2017). Also, this group of orchids is frequently used in the pharmaceutical and cosmetic industries and medicine. Species of the genus *Epipactis* and *Spiranthes* have been used to treat various diseases (Lans, 2006; Peng, 2007). Some terrestrial orchid species (e.g., species of the genus *Gymnadenia*) produce a fragrance that contains chemical compounds used in perfumes, aromas, and essential oils, such as benzyl acetate, benzaldehyde, and eugenol (Huber et al., 2005; Schiestl et al., 2011). According to Kreziou et al. (2016), the use of orchids is of great economic importance at both the local and national levels. However, the uncontrolled and illegal collection of terrestrial orchids from their natural populations poses a significant threat to their survival, mainly due to the very complex and lengthy reproduction process.

Orchids have a very high conservation value worldwide due to their complex relationship with other organisms - the obligatory establishment of symbiosis with certain fungi and the need for certain groups of insects for pollination (Swarts & Dixon, 2009). However, there is still a significant decline in the number and size of orchid populations in their natural habitats, particularly in the last 30 years (Seaton et al., 2010).

Figure 4: Species commonly used in salep production: a) *Orchis purpurea*, b) *Orchis militaris*, c) *Orchis mascula*, d) *Neotinea tridentata* (Original by Ostojić)

Small populations have an increased risk of extinction due to different genetic drift types and inbreeding, which may have a negative impact on the fitness of individuals or populations (Lienert, 2004). This fact is particularly reflected in the case of terrestrial orchids, as they require a specific group of insects for pollination, which leads to reduced fertilization, reduced fruit set, and population growth, while at the same time favoring inbreeding depression (Cribb et al., 2003). Some populations of terrestrial orchids move away from each other (Dulić et al., 2020), resulting in reduced pollination and fertilization, which has a negative effect on the fruit set and seedling recruitment (Jacquemyn et al., 2006). These small and fragmented populations are also exposed to negative biotic and abiotic factors. Climate change significantly affects the performance of orchids, which is reflected in their distribution and population dynamics and a disturbed co-evolutionary plant-pollinator relationship due to global warming (Robbirt et al., 2014). For example, the species *O. sphegodes* has a flower similar to the female bee *Andrena nigroaenea*, and pollination is by pseudocopulation. At slightly higher spring temperatures, the bees started their mating cycle before the flowering of this species, which resulted in no fertilization and no fruit set. Moreover, some ecosystems are likely to be more vulnerable to climate change than others (Seaton et al., 2010). The Fruška Gora Mountain (Province Vojvodina - North Serbia), with 32 orchid species (Savić, 1998), could be an example of the negative impact of global warming on the distribution of terrestrial orchids. The mean annual soil temperature in Vojvodina could increase by up to 3.5°C in the 0-10 cm superficial layer (Ćirić et al., 2017). As this soil layer is the root system zone for terrestrial orchids, this temperature increase could lead to disturbances in nutrient storage, plant growth, and organ formation in the following years and decades (Dulić et al., 2020). In contrast, mild warming could positively affect the distribution of *Orchis anthropophora*, *Orchis purpurea*, *Orchis simia* (Evans et al., 2020), *Himantoglossum hircinum,* and *Ophrys sphegodes* (Kull & Hutchings, 2006). However, sustained warming could be disadvantageous, especially in the lower latitudes, if the temperature rise exceeds 4°C (Evans et al., 2020). Although climate change affects the distribution of terrestrial orchids, anthropogenic influences such as uncontrolled collection and destruction of both the above-ground parts during flowering and the underground organs used later in the processing are much more vulnerable (Cribb et al., 2003; Swarts & Dixon, 2009). Also, the uncontrolled transformation of meadows and forests, which are natural habitats of terrestrial orchids, into arable land and hut settlements, while many are used as pastures for livestock (Figure 5) (Swarts & Dixon, 2009; Paul et al., 2013).

Consequently, most terrestrial orchids are protected by international or national institutions and are listed in various directives and IUCN Red Book (Dumant et al., 1996; Whigham & Willems, 2003). Due to the very pronounced adverse anthropogenic effects, in which the illegal distribution of tubers is particularly marked, all orchid species have been included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The orchid species listed by convection account for 9.8% of Appendix I and 73% of Appendix II and comprise 70% of the total CITES species covered by the Convention (CITES, 2013).

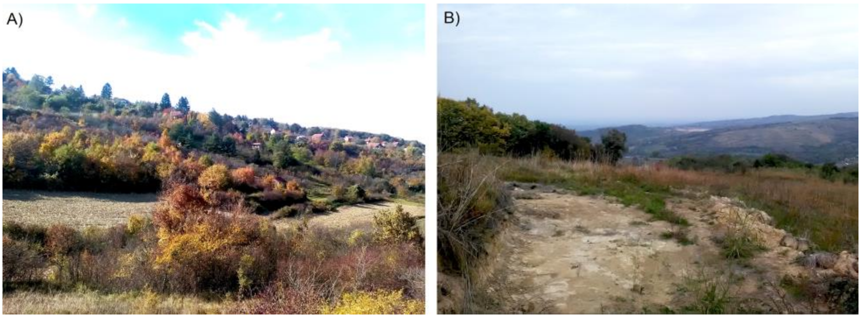

Figure 5: Disturbed natural habitats of terrestrial orchids in Fruška Gora Mountain, a) habitat of several orchid species, surrounded by arable land and hut settlements; b) degraded habitat of *Orchis purpurea* and *Gymnadenia conopsea* (Original by Ostojić)

Consequently, most terrestrial orchids are protected by international or national institutions and are listed in various directives and IUCN Red Book (Dumont et al., 1996; Whigham & Willems, 2003). Due to the very pronounced adverse anthropogenic effects, in which the illegal distribution of tubers is particularly marked, all orchid species have been included in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). The orchid species listed by convection account for 9.8% of Appendix I and 73% of Appendix II and comprise 70% of the total CITES species covered by the Convention (CITES, 2013).

3.2. Application of *In Vitro* Techniques

3.2.1. Support to Sustainable Use and Conservation of Natural Terrestrial Orchid Populations

The current overexploitation of natural terrestrial orchid resources and widespread land degradation, leading to a decline in terrestrial orchid populations, urge for the development of an effective integrative approach to combat the threats and minimize the harmful effects. Integrated approaches to the conservation of endangered species are based on the networking of ecological and genetic research as well as *in situ* practices and *ex situ* reproduction of essential material (Ramsay & Dixon, 2003). The propagation of plant material using protocols under controlled *in vitro* conditions has proven to be a sustainable method for propagating rare and endangered plant species for the purpose of their conservation and wider application (Fay, 1994). *In vitro* technologies supporting *ex situ* conservation, such as symbiotic and asymbiotic seed germination, micropropagation, and cryopreservation, are most commonly used for conservation of rare and endangered plant species, especially orchids (Fay, 1994; Stewart & Kane, 2006a,b; Dutra et al., 2008; Bhattacharyya et al., 2017). The two techniques, symbiotic and asymbiotic seed germination, have their challenges reflected above all in species-specific requirements for the environmental conditions that must be met in the culture. For this purpose, individual protocols must be established for each species, whereby the appropriate technique (symbiotic or asymbiotic germination) must first be determined. Although many improvements have been made to both techniques in recent years, particularly in terms of the number of species successfully introduced into a culture, there are still some aspects that should be addressed. Protocols based on symbiotic seed germination require further studies on orchid-fungal interactions, the metabolism of the fungal partner, inoculation conditions, and evaluation of the increment of application of the fungi in axenic seedlings. Similarly, further scientific research in asymbiotic seed germination should be focused on establishing protocols adapted to each species individually, which would have a conservational and economic impact and may also be important for breeding research. Established and improved *in vitro* germination protocols can be applied to the *ex situ* conservation of endangered species and populations. The propagated material can be used for the reintroduction, restoration, and maintenance of stable populations *in situ*. In this context, the efficiency of the techniques used (symbiotic or asymbiotic) in terms of survival rates in the field should be further evaluated. In addition, the techniques could be used in the mass propagation of species of interest to the food, pharmaceutical and cosmetics industries, which could considerably reduce the pressure on natural populations.

The development of a database containing all existing guidelines and protocols for *in vitro* seed germination of terrestrial orchids would facilitate access to the studies carried out, which could further reduce the time needed for protocol design for each species. The database could be used to test existing protocols assigned to species with the same or even similar environmental requirements, taxonomic affiliations, or fungal partners before focusing on the development of new protocols. In addition, easy access to existing protocols would facilitate the propagation of terrestrial orchid species outside the scientific framework, thus opening up the field of commercial propagation of terrestrial orchids as ornamentals. Consequently, market demand could further support progress in the *in vitro* propagation of terrestrial orchids.

3.2.2. Commercialization of Terrestrial Orchids as Ornamentals

Some of the key requirements, such as novelty, uniqueness, distinctness, stability, and necessity, should be fulfilled to penetrate the market successfully (Nau, 2007). A recent study on the marketability potential of terrestrial orchids has shown that the species *Spiranthes spiralis*, *Orchis purpurea*, *Orchis militaris*, *Himantoglossum jankae*, *Anacamptis pyramidalis*, *Neotinea tridentata*, *Platanthera bifolia*, *Limodorum abortivum*, *Orchis mascula,* and *Epipactis helleborine* fulfill the requirements and therefore have great potential as a new product in the horticultural market (Dulić et al., 2020). The development of easy performable and effective symbiotic and asymbiotic protocols would further facilitate the penetration to the market.

The technique of symbiotic seed germination can be applied in the commercial production of plants (Markovina & McGee, 2006). However, more knowledge about the fungal partner in terms of its metabolism and preservation and the potential for mass production and commercialization of fungal isolates is needed to promote this technique in the commercial propagation and mass production of terrestrial orchids. At present, asymbiotic seed germination is more of a technique of choice because of its cheaper, more accessible, and faster method, although a separate protocol needs to be developed for each species. However, even in commercial production, the efficiency of both techniques should be evaluated by the survival rate of individuals after field introduction to confirm the stability and quality of the product. From an economic point of view, the protocols obtained should form the basis for a mass production that would allow the penetration of terrestrial orchids on the horticultural market and their wider use as ornamental products for various purposes. Mass production would also provide source material for the selection of parent combinations for planned crosses and the creation of genotypes suitable for use in horticulture and landscape architecture.

CONCLUSION

The development of propagation techniques for the *ex situ* germination of terrestrial orchids offers the chance to improve the condition of endangered populations of terrestrial orchids. Both symbiotic and asymbiotic techniques complement *ex situ* conservation practices by providing the plant material to enrich and refresh affected populations and reintroduce them into habitats from which they have disappeared due to overexploitation and land degradation.

These techniques could make it possible to propagate on a large scale some species that are intensively collected in natural habitats for the needs of the food, pharmaceutical, and cosmetic industries. Simultaneously, these techniques will allow a stable introduction of terrestrial orchids as ornamental species on the horticultural market. Further development of the techniques is necessary, and this is primarily related to the study of host-fungal interaction and the development of protocols for as many species as possible. An important contribution to further research would be a database containing all protocols developed so far. A single database would significantly reduce the time needed to develop protocols for new hybrids or species close to species for which a propagation protocol has already been developed.

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CHAPTER 18

A REVIEW; EVALUATION OF PLANT GROWTH PROMOTING RHIZOBACTERIA IN ORNAMENTAL PLANTS INDUSTRY

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INTRODUCTION

Ornamental plants can directly affect on the mental and psychological state of people due to its shape, beauty, color, texture and scent and play an important role in the afforestation of cities and the beautification of indoor and outdoor spaces. Ornamental plants are an industry worth billions of US dollars annually worldwide and have a large market (Tao et al., 2015). Ornamental plants serve as the main source of export materials globally and add value to the country's economy. In addition, ornamental plants are not only used for aesthetic purposes but some ornamental plants also are used to combat certain human diseases and have medicinal value. For example, tea of yellow and white chrysanthemum flowers is used against to flu (Karishma et al., 2013; Zaidi et al., 2016). When outstanding and diverse importances of ornamental plants is considered, there is a need to improve the flower quality characteristics of these plants, such as growth and development, flower yield, number, longevity and size, and the aesthetic properties of indoor and outdoor plants.

To produce high quality ornamental plants, growers have generally concentrated on the use of chemicals (including pesticides and fertilizers) used in agriculture, without taking into account the ornamental plant characteristics and their harmful effects on the environment. In addition, agricultural chemicals used in the cultivation of ornamental plants are expensive and their excessive usage leads to the emergence of pathogens resistant to such that of these chemicals.

Various synthetic fertilizers such as nitrogen, potassium and phosphorus are used at high rates for quality and efficient production in ornamental plants providing a significant financial benefit to growers. In general, the main reasons for fertilizing ornamental plants are to stimulate growth or to obtain strong, healthy, highly quality and aesthetic plants. The excessive cost of chemical fertilizers and the toxic effect of such chemicals on soil microflora and indirectly on human health, are the biggest concerns for growers (Younis et al., 2013). In addition, long-term fertilizer application also destroys the soil structure (Singh et al., 2008) and thus affects on the production and growth of ornamental plants (Mohammadi Torkashvand, 2009). Therefore, it has become necessary to develop renewable, cheap and environmentally-friendly fertilizers without impairing the quality of ornamental plants.

In this regard, rhizobacteria that support plant growth have been identified as an effective alternative to agricultural chemicals and chemical fertilizers in the cultivation of ornamental plants. Plant growth promoting rhizobacteria (PGPR), which is a substance that contains live microorganisms that colonize the plant rhizosphere or interior when applied to the soil or seed and plant surfaces, and promotes growth by increasing in the supply or availability of the plant, which is often called to as bio fertilizers, has provided solid solutions to toxic chemicals in sustainable plant production (Zawadzka et al., 2013; Karpagam & Nagalakshmi, 2014; Zaidi et al., 2016; Gouda et al., 2018).

Although there are sufficient reports on the effect of PGPR on the growth and development of a large number of plants, information on the effect of PGPR on the quality and production of ornamental plants are limited (Zulueta-Rodriguez et al., 2014: Parlakova Karagöz et al., 2016). Considering the achievements to date of PGPR applications in horticultural plants (Botta et al., 2013; Arikan & Pirlak, 2016; Pii et al., 2017; Pahari et al., 2017; Kutlu et al., 2019), PGPR in different production systems, studies have been started to emphasize the effect on the production of different grown ornamental plants (Sachin & Misra, 2009; Mishra et al., 2010; Koley & Pal, 2011; Abbasniayzare et al., 2012; El-Deeb et al., 2012; Raval & Desai, 2012; Prasad et al., 2012; Ashwin et al., 2013; Karishma et al., 2013; Chaudhari et al., 2013; Qasim et al., 2014; Hoda & Mona, 2014; Kumari et al., 2014; Srivastava et al., 2014; Ramlakshmi & Bharathiraja, 2015). Additionally, the role of PGPR in the management of ornamental plant diseases was discussed and evaluated (Chandel & Deepika, 2010; Orbera´ et al., 2014; Postma et al., 2013; Sivasakthi et al., 2013; Zaidi et al., 2014; Zaidi et al., 2016). Generally, PGPR facilitates the growth of ornamental plants by providing essential nutrients (Abbasniayzare et al., 2012). It improves the quantity and quality of ornamental plants (Karishma et al., 2013). For example, bio fertilizers increased in plant height, tepal diameter, leaf area and tulip flower growth and quality, and also increased in bulb yield (Khan et al., 2009). Another for example, Rajesh et al. (2006) also found that *Azospirillum* and *Bacillus subtilis* increase in the fresh weight of the flower in clove. Parlakova Karagöz & Dursun (2019a) stated that the bacterial formulation consisting of *Pantoea* *agglomerans, Pantoea agglomerans, Bacillus megaterium, Paenibacillus polymyxa* bacteria isolates increased in the number of bulblets in tulip. The use of PGPR alone or in combination with fertilizers to improve the physico-chemical and biological properties of the soil is also one of the methods used in plant cultivation. As a result, efforts are made to increase in product yield (Baloach et al., 2014). Various research results show that the combinations of PGPR + AM fungi (Ramlakshmi & Bharathiraja, 2015), PGPR + endophytes, PGPR + vermicompost (Narayanagowda, 2003; Karuppaiah, 2005; Nazari et al., 2008; Pandey et al., 2017; Parlakova Karagöz et al., 2019a), PGPR + chemical fertilizer (Parlakova Karagoz & Dursun, 2020a,b,c) can be used very effectively to increase in the flower quality and yield of ornamental plants.

Recent research has shown that varieties of ornamental plants grown in greenhouses under normal conditions can increase in plant quality by increasing plant size and number of flowers when grown with different PGPR applications, even when exposed to abiotic stress (Flores et al., 2007; Nordstedt et al., 2020). However, to positively affect on the quality of plants exposed to abiotic stress, PGPR firstly induces physiological changes increasing in the plant's stress tolerance and allow the plant to better overcome stress during recovery (Yang et al., 2009). Secondly at the same time, PGPR positively affects on plant resilience and plant growth, resulting in a higher quality product that is more tolerant to nutrient-deficient and drought conditions that may be encountered during retail and shipping, to abiotic stress after production (Ruzzi & Aroca, 2015).

If PGPR-plant interactions are indeed identified and applied appropriately, reducing soil pollution from excessive fertilizer application and preserving soil fertility will be achieved. And as a result, PGPR can contribute greatly to ornamental plant growers in increasing yield and various ornamental plant characteristics. Although there are extensive reports on the effect of PGPR on the growth and development of different plants (Panhwar et al., 2014; Arab et al., 2015; Parlakova Karagöz et al., 2016; Bahadır et al., 2018; Seema et al., 2018; Kenneth et al., 2019; Alalaf, 2020), information on the effect of PGPR on the quality and production of ornamental plants compared to other crops to date is insufficient. With this gap in mind, the role of PGPR in ornamental plant production is once again discussed and highlighted in this review.

1. PGPRs AND THEIR MODE OF ACTION

PGPRs are microorganisms promoting seed germination, have a role in accelerating growth and increase in crop yield, can produce physiological active substances in the ability to tolerate drought, provide resistance to certain diseases, and improve plant defense against to pathogens (Glick et al., 1999; Choudhary et al., 2019). Also, rhizobacteria play an important role in regulating the overuse of fertilizers and pesticides (Ashrafuzzaman et al., 2009; Dalve et al., 2009; Mishra et al., 2010; Parlakova Karagöz & Dursun, 2019b,c).

PGPR is generally grouped as biofertilizer that use to increase in the nutrient content of plants, plant stimulants promoting plant growth and development by plant hormone synthesis, biopesticides using to control diseases or promote systemic resistance in plants because of their competitive properties with antibiotic and antifungal metabolite production and rhizoremeditors using to break down organic pollutants (Hecht-Buchholz, 1998; Amer & Utkheda, 2000; Khalid et al., 2004; Antoun & Prevost, 2006).

Among the rhizobacteria group accepted as PGPR, there are generally *Acinetobacter, Agrobacterium, Bacillus, Aereobacter, Achromobacter, Alcaligenes, Azospirillum, Artrobacter, Enterobacter, Burkholderia, Microccocus, Xanthomonas, Flavobacterium, Erwinia, Pseudobium, Serizobacterium* (Montesinos et al., 2002; Goswami et al., 2016). Beneficial bacteria are also used as symbiotic (*Mesorhizobium, Bradyrhizobium, Rhizobium*) and non-symbiotic (*Azotobacter, Pseudomonas, Acotobacter, Klebsiella, Bacillus, Azomonas, Azospirillum*) agents (Saharan & Nehra, 2011) to increase in plant growth under biotic stress conditions (El-Meihy, 2016; Salman et al., 2017; Seymen et al., 2019). Some strains of bacteria directly regulate plant physiology by mimicking the synthesis of plant hormones, while others increase in the availability of nitrogen and mineral in the soil as a means of enhancing growth (Yasmin et al., 2007); Some types of bacteria also include mycorrhiza helper bacteria that help arbuscular mycorrhizal fungi (AMF) (Andrade et al., 1997; Moncada et al., 2020). The effects of the bacteria promoting plant growth may vary depending on the bacteria-plant combination, bacterial type and number, growth period, plant genotype, harvest date, soil type, vegetative parameters, organic matter amount and environmental conditions (Çakmakçı et al., 2006). Root secretions as inoculants have also been reported to play a central role in the effectiveness and success of PGPR. Plant roots produce a fairly unlimited variety of compounds in response to prevailing factors in their habitat, including both abiotic and biotic factors; this effect ultimately affects on the plant-bacteria interaction.

Zahir et al. (2004) stated that changing the rhizosphere increases in health of the plant and the production by supplementing or replacing the resident microflora with beneficial microorganisms. As a result of many studies, it has been reported that some of the many bacteria found in the rhizosphere of the plant roots have effects on increase in the vegetative and generative growth and development of plants (Rodriguez & Fraga, 1999; Sudhakar et al., 2000; Vessey, 2003; Niranjiyan Raj et al., 2006; Eşitken, 2011). PGPR are free-living microorganisms that beneficially colonize the surface of plant roots (Woitke & Schitzler, 2005) and are known to affect on plant growth through a variety of direct or indirect mechanisms (Heidari & Golpayegani, 2012; Choudhary et al., 2019).

PGPRs are generally divided into two classes according to their effectiveness. PGPRs that are directly effective in plant growth and development, seed germination and emergence, yield of products, and PGPRs that can be indirectly beneficial to plant growth and development through bio-control (Glick et al., 1999; Choudhary et al., 2019). PGPRs directly affect on the growth and development of the plant by improving the intake of iron and some other micro elements, especially with the production of siderophore and organic acids, by fixing the nitrogen in asymbiotic form in the growing medium, accelerating the solubility of organic phosphorus and inorganic phosphorus compounds. PGPRs can be directly increased in plant growth and development with production of herbal hormones (such as cytokines, auxins, gibberelins), by synthesizing ACC-1-aminocyclopropane- 1-carboxylate deaminase enzyme (Jacobson et al., 1994), it hydrolyzes ethylene biosynthesis in plants at the rhizospheric level and provides its reduction, by reducing environmental stress, by adaptation in plant-bacteria relationship, by increasing in plant root permeability, by synthesizing vitamins (Zahir et al., 2004; Çakmakçı et al., 2006; Aslantaş et al., 2007; Çakmakçı et al., 2007; Akgül & Mirik, 2008). With these properties, PGPRs are effective in cell division and expansion (Taiz & Zeiger, 2002); they stimulate the development of the plant's organs by improving the intake of plant nutrients (Yanni et al., 1997). PGPRs contribute to plant growth and development to thanks to all these mechanisms such as root growth and increase in weight, yield, seed germination rate, chlorophyll content, leaf area, N, Mg, P content, protein ratio, drought tolerance, hydraulic activity, shoot weights, formation of the abscess layer on the leaf (Dobbelaere et al., 2003; Lucy et al., 2004; Çakmakçı, 2005a,b). The indirect effects of PGPRs are to increase in plant resistance, preserve it, accelerate plant growth and development, or ensure that the plant is less affected by the harmful effects of pathogens through the secretion of inhibitory substances. In addition, they have been reported to reduce plant diseases by producing antibiotics as a bio-control agent. It is stated that they break down the inhibiting xenobiotics in the growing environments contaminated with many organic compounds and thus protect the plants (Elsheikh & Elzidany, 1997; Rodriguez & Fraga, 1999; Fernando et al., 2006). The functioning of the biocontrol mechanism of PGPRs is provided by the synthesis of antibiotics such as Oomycin, 2,4 diacetyl phloroglucinol, Phenazine-1-carboxylic acid, Pyrrolnitrin, Pyuloteorin, Kanosamine, Pantocin and Zwittermycin-A (Fernando et al., 2006). These antibiotics have a wide range of activities and the most effective and most studied is 2,4 diacetyl phloroglucinol (Raaijmakers et al., 2002; Arıkan, 2012). They can also be used as bio fertilizers as they can produce enzymes to dissolve mineral substances, act as biocontrol agents and biological fungicides (Karpagam & Nagalakshmi, 2014). Some bacterial strains such as *Bacillus, Pseudomonas, Arthrobacter, Serratia* and *Stenotrophomonas* stimulate plant growth by promoting volatile organic compounds. Acetone and 2, 3-Butanediol produced by *Bacillus* spp. stimulate plant growth by suppressing fungal reproduction (Seymen et al., 2019).

2. EFFECT OF PGPR ON ORNAMENTAL PLANTS

The development of healthy ornamental plants, increasing in their production and quality are some of the important issues that require special attention in commercial ornamental plant technology. To achieve these aims, expensive and environmentally damaging chemical fertilizers are extensively used. However, the inoculation of PGPR plays an important role in reducing the application of inorganic fertilizers, as well as increasing in the quality and yield of different ornamental plants, maintaining soil fertility (Qasim et al., 2014).

The effects of *B. subtilis* (MA-2 strain) and *P. fluorescens* (MA-4 strain) rhizobacteria applications on *Geranium* were investigated. As a result of this research, it was found that rhizobacteria applications increased in the plant yield (Mishra et al., 2010) (Table 1, Figure 1 [1]).

Figure 1: [1]; Effect of PGPR on the growth of *Pelargonium graveolens* (A) *Bacillus subtilis* (B) *Pseudomonas fluorescens* (C) Control (Mishra et al. 2010), [2]; Parlakova Karagöz et al. (2016) view from harvest and measurement evaluation parameters of safroon corms

In a follow up study, the effect of *A. chroococcum* on seedling growth of bamboo (*Bambusa bamboo*) has been investigated. A significant increase was observed in root and shoot lengths and dry matter accumulation of seedlings harvested 25 days after growth. The researchers explained these increases as the ability P solubility and produce IAA of *A. chroococcum*. As a result of the research, it has been suggested that using *A. chroococcum* in bamboo cultivation may be beneficial (Dhamangaonkar & Misra, 2009) (Table 1).

This study was carried out to determine the effects on the growth and yield enhancement of *Eleusine coracana* and *Amaranthus paniculatus* plants growing at higher altitudes, of two isolates of *Pseudomonas corrugata* 1 and *Pseudomonas corrugata* 7. It was determined that *Pseudomonas corrugata* stimulated the root colonization. In general, it has been noted that various physiological characteristics and the original habitat of the microbe should be considered while selecting an inoculant. In addition, it has been determined that plant-microorganism compatibility is important for the establishment of bacterial isolates in their original habitat and has a sufficient effect on plant growth (Pandey et al., 1999). Ten different applications were evaluated in the study conducted with the aim of increasing in the yield and quality of saffron corms and expanding their production as an ornamental plant. At the end of the experiment under greenhouse conditions, growth and yield values of saffron were obtained higher or equal in some bacterial applications than hormone applications. It was concluded that the biofertilizers used in organic agriculture have a positive effect on the increase in saffron plant growth and development (Parlakova Karagöz et al., 2016) (Table 1, Figure 1 [2]).

In a study, which investigated the role of rhizobacteria promoting plant growth for growth and rooting in *Rosa damascena* Mill, different fifteen starins that selected from fast growing rhizobacterial colonies (strains) selected were inoculated on Rosa cuttings by dipping method.

Significant results were found in growth parameters. Cutting mortality rate was determined to be insignificant. As a result of potting trials, B5 has proven to be a growth limiting strain as most isolates yield 20-40% less results. With the exception of the B5 isolate, other isolates were demonstrated to show growth promoting activities in *Rosa* cuttings for all parameters. The most significant results were obtained from isolated strains LSI19 (*Rhizobium leguminosarum*), F6 (*P. fluorescens*) and LC4 (*V. vulnificus*). Isolate LSI19 increased in new shoot fresh and dry weights, shoot length by 51.6%, 55.2% and 48.0%, respectively, compared to the uninoculated control. Root growth, root length and root fresh and dry weights parameters were positively affected by the F6 isolate (Tariq et al., 2016) (Table 1, Figure 2).

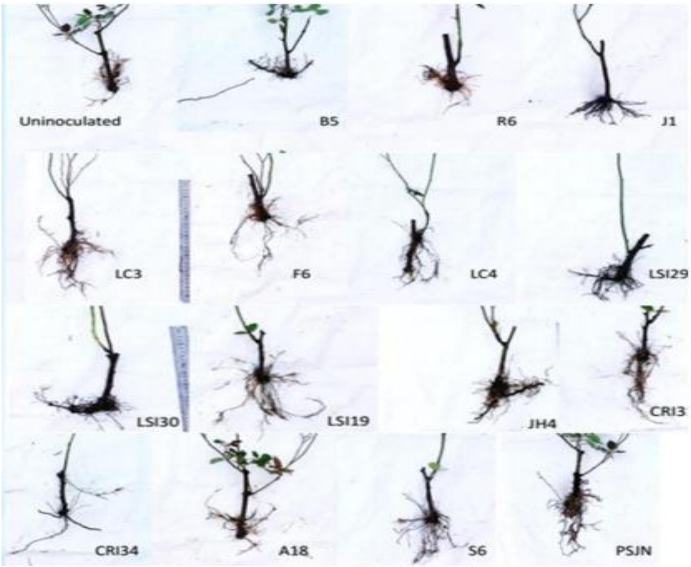

Figure 2: Pictorial view of root length of cuttings by PGPR strains application (Tariq et al., 2016)

Bacillus licheniformis MH48 rhizobacterium was used to promote plant growth in the cultivation of seedlings of *Camellia japonica* ornamental plants in the improved coastal lands. As a result, it has showed that inoculation with *B. licheniformis MH48* can be used as a PGPR bioenhancer to stimulate fine promote nutrient uptake, alleviate salt stress and root growth in ornamental plant seedlings grown in high salinity conditions of reclaimed coastal land (Park et al., 2017) (Table 1).

The size of the bulbs is directly proportional to the quality of the flower, the commercial value of the bulb and the more bulb yield. The study was carried out to evaluate the effects of PGPR on plant growth parameters, flowering, bulb quality and bulb mineral contents in hyacinth (*Hyacinthus orientalis* L. cv. Aiolos) under greenhouse conditions. Chlorophyll content, leaf length, leaf area, fresh and dry weight of flowers, bulb diameter, bulb length and bulb weight were found to be maximum at T2 (*Kluyvera cryocrescens* RCK-113C). The highest leaf width and flower number were obtained from T4 (*Bacillus subtilis* strain RCK-17C). Maximum N, P and Ca were found at T3 (*Paenibacillus polymyxa* strain RCK-12E). At the end of the study, it was concluded that the use of bacteria isolates of *Kluyvera cryocrescens* strain RCK-113C and *Pseudomonas putida* strain RCK-42A may be effective in maintaining the sustainability of the environment and growing environment in hyacinth cultivation (Parlakova Karagöz et al., 2019b) (Table 1).

Table 1: Examples of PGPR inoculation on plant growth and yield components of some ornamental plants

Ornamental plant material	Botanical name	Inoculant PGPR	Comments	References
Geranium	*Pelargonium graveolens* L. Herit	*Bacillus subtilis* (MA-2) and *Pseudomonas fluorescens* (MA-4)	Yield	Mishra et al., (2010)
Bamboo	*Bambusa bamboo*	*Azotobacter chroococcum*	Seedling growth and dry matter accumulation	Dhamangaonkar & Misra (2009)
Chua/ chauli and madua	*Amaranthus paniculatus* and *Eleusine coracana*	*Pseudomonas corrugata*, *P. corrugata* 1, *P. corrugata* 7 and *Azotobacter chroococcum*	Plant growth, nitrogen content and root colonization	Pandey et al., (1999)
Saffron	*Crocus sativus* L.	*Bacillus* GC group B strain TV119E, *Brevibacillus choshinensis* strain TV-53D, *Achromobacter xylosoxidans* strain TV-42A, *Myroides odoratus* strain TV-85C, *Colwellia psycrerytreae* strain TV-108G, *Bacillus megaterium* strain TV-87A and *Kluyvera cryocrescens* strain TV113C	Corm yield, plant growth and development	Parlakova Karagöz et al., (2016)
Rosa	*Rosa canina*	*Bacillus megaterium, Pseudomonas fluorescens*	Rooting rate	Kınık, (2014)
Weeping fig / Ficus	*Ficus benjamina* L.	*Bacillus subtilis* *Pseudomonas putida*	Root formation and the number of leaves	Sezen et al., (2014)
Rosa	*Rosa damascena* Mill.	F6 (*Pseudomonas fluorescens*), LSI19 (*Rhizobium leguminosarum*) and LC4 (*Vibrios vulnificus*)	Shoot and root growth characters	Tariq et al., (2016)
Camellia	*Camellia japonica*	*Bacillus licheniformis* MH48	Nutrient intake, auxin	Park et al., (2017)
Hyacinth	*Hyacinthus orientalis* L. cv. Aiolo	*Pseudomonas putida* strain RCK-42A, *Kluyvera cryocrescens* strain RCK-113C, *Paenibacillus polymyxa* strain RCK-12E, *Bacillus subtilis* strain RCK-17C	Plant growth parameters, flowering, bulb quality and bulb mineral contents	Parlakova Karagöz et al., (2019b)

Formulation is a very important issue for inoculants containing an effective bacterial strain. It can determine the success or unsuccessful of a biological agent. The use of inoculant formulations containing carrier materials for introducing microbial cells to the soil or to the rhizosphere is an attractive and effective option (Latha et al., 2009). In this part of the review, the studies on the application of PGPR formulation on ornamental plants were evaluated.

In a study investigating the effect of three different *P. putida* strains and their mixtures on two varieties of poinsettia *(Euphorbia pulcherrima*), an increase in plant growth and anthocyanin pigmentation was found. It was determined that the effect of *P. putida* significantly affected on features such as cyathia number, root volume, leaf number, leaf area, when compared to the control. It has also been reported that *P. putida* is important in plant's bracts coloration (Zulueta-Rodriguez et al., 2014) (Table 2).

The use of *Bacillus megaterium, Pseudomonas fluorescens* bacteria in *Rosa canina* wea constituted the highest rooting rate (Kınık, 2014). In *Ficus benjamina* L., *Bacillus subtilis* bacteria promoted root formation and *Pseudomonas putida* bacteria increased in the number of leaves (Sezen et al., 2014) (Table 2).

In order to evaluate the potential to be growth promoters of *Pseudomonas putida* rhizobacteria species, the rhizosphere region of Christmas Eve variety of poinsettia was inoculated with the solutions of *P. putida*'s FCA-8, FCA-60 and FCA-56 strains. Applications as control, FCA-8, FCA-60, FCA-56 and their combination were designed and inoculated onto plants; plants were fertilized with 50% of the dose of chemical fertilizers commonly used in the production of these plants. All rhizobacterial strains increased in the weight of fresh and dry leaves and promoted the growth and development of plants. It was found that the FCA-8 strain gave the best results. Strain FCA-56 strain gave the best results for fresh weight of bract leaves, dry weight of root and stem diameter. The results have showed that *P. putida* rhizobacteria's potential as a growth promoter and an alternative supporting the reduction in the use of chemical fertilizers (Silva & Iveth, 2011) (Table 2).

Bulbs of three different tulip cultivas (Pink Impression, Blue Aimable, and Golden Parade) belonging to the *Tulipa gesneriana* L. was inoculated with four different bacterial formulations. According to the research findings, important results were obtained between applications and varieties. As a result of the study, it was concluded that the number and quality of bulblets can be increased in bacterial formulation applications, especially the application of formulation C (RK-79 + RK-92 + TV-3D + TV-12E) (Parlakova Karagöz & Dursun, 2019a) (Table 2) (Figure 3).

Different PGPR formulations have been injected into the root region. Three different tulip varieties belonging to *Tulipa gesneriana* L., which are widely used in parks and gardens in some country in all araund of the word, were used. The study consisted of 5 treatments. It has been determined that there is a general interaction of "Type x applications".

Figure 3: Pictorial view of *Tulipa gesneriana* L. cv. Golden Parade by PGPR strains application (Original by Parlakova Karagöz)

The highest total nitrogen (2.53%) and P (0.34%) amounts were determined in the bulbs of the Pink Impression variety in Formation C according to the control application. It has been determined that Formulation C (*P. agglomerans* RK-92 + *P. agglomerans* RK-79 + *B. megaterium* TV-3D + *Paenibacillus polymyxa* TV-12E) and Formulation D (*P. agglomerans* RK-79 + *P. agglomerans* RK-92 + *B. megaterium* TV-6D + *Pseudomonas putida* TV-42A) applications in particular have important effects on bulb, leaf and soil nutrient element content (Parlakova Karagöz & Dursun, 2019d) (Table 2).

A research was conducted by Parlakova Karagöz (2020) to examine the effects of different doses of the bacterial formulation, which consists of a mixture of *Kluyvera cryocrescens* TV-113C, *Bacillus megaterium* TV-91C and *Pantoea agglomerans* RK-92 strains, on plant growth and quality of poinsettias. Each of the solutions containing bacterial suspensions 52.5 (T1), 105 (T2), 210 (T3), 420 (T4) and 840 (T5) $mL \cdot L^{-1}$ of water was applied to the plant rhizosphere. This study revealed positive changes in plant and bract parameters with increasing doses of bacterial formulation in poinsettia plants. This effect reached the maximum level in the parameters of bract number, bract diameter, main flower stem length, plant fresh weight, nitrate reductase enzyme activity and maximal root length, with the T5 treatment, with the highest concentration of bacterial formulation (Figure 4).

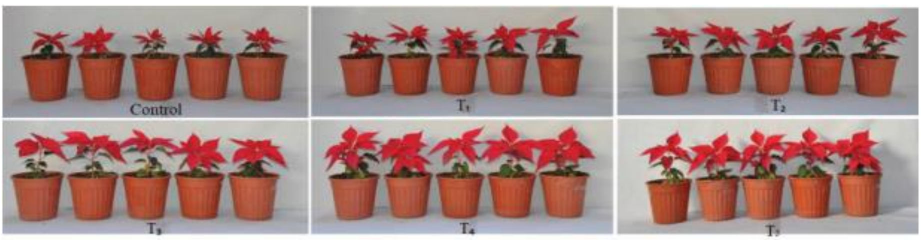

Figure 4: The appearance of all treatments and control plants on day 160 (Original by Parlakova Karagöz)

Bio fertilizers are products that contain living cells of different microorganism species and have the ability to transform nutrient-important elements into non-existing form by biological processes (Vessey, 2003; Wu et al., 2005). In recent years, bio fertilizers have been an important component of an integrated nutrient supply system and environmentally more It makes a great promise in improving crop yields through good food sources (Wu et al., 2005).

Table 2: Examples of PGPR applied in combination on plant growth and yield components of some ornamental plants

Ornamental plant material	Botanical name	Inoculant PGPR	Comments	References
Poinsettia	*Euphorbia pulcherrima* Willd. Ex Klotzsch	Three different *P. putida* strains and their mixtures	Plant growth and anthocyanin pigmentation, cyathia number, leaf number, leaf area, plant's bracts coloration	Zulueta-Rodriguez et al., (2014)
Christmas Eve variety of poinsettia	*Euphorbia pulcherrima*	*Pseudomonas putida*'s FCA-8, FCA-60 and FCA-56 strains	Growth and development of plants	Silva & Iveth, (2011)
Tulip	*Tulipa gesneriana* L.	Four different bacterial formulations including *Pantoea agglomerans RK-79, Pantoea agglomerans RK-92, Bacillus megaterium TV-91C, Bacillus subtilis TV-17C, Bacillus megaterium TV-3D, Paenibacillus polymyxa TV-12E, Bacillus megaterium TV-6D, Pseudomonas putida TV-42A* bacterial strains	Number of main bulb, number and quality of bulblets	Parlakova Karagöz & Dursun, (2019a)
Tulip	*Tulipa gesneriana* L.	Formulation A (*Pantoea agglomerans* RK-79 + *Pantoea agglomerans* RK-92), formulation B (*P. agglomerans* RK-79 + *P. agglomerans* RK-92 + *Bacillus megaterium* TV-91C + *Bacillus subtilis* TV-17C), formulation C (*P. agglomerans* RK-79 + *P. agglomerans* RK-92 + *B. megaterium* TV-3D + *Paenibacillus polymyxa* TV-12E), formulation D (*P. agglomerans* RK-79 + *P. agglomerans* RK-92 + *B. megaterium* TV-6D + *Pseudomonas putid*a TV-42A)	Bulb, leaf and soil nutrient element content	Parlakova Karagöz & Dursun, (2019d)
Poinsettia	*Euphorbia pulcherrima* Willd. Ex Klotzsch	Mixtures of *B. megaterium* TV-91C, *P. aglomerans* RK-92 and *K. cryocrescens* TV-113C strains and their diffrent levels	Plant growth and quality of bracts	Parlakova Karagöz, (2020)

There is great potential for use of PGPR as bio-fertilizing agents for a wide variety of crop plants in a wide variety of edaphic and climatic conditions (Lucy et al., 2004). Hashemabadi et al. (2012) evaluated the effect of Barvar-2 bio fertilizer containing of *Bacillus lentus* and *P. putida* (Raissi et al., 2013) and different doses of phosphorus on various characteristics of marigold. As a result of the general evaluation, the interactive effect of bio fertilizer and P was not statistically significant for other parameters measured except for the total P accumulation on the shoot and the number of leaves per plant. Zaredost et al. (2014) also conducted a similar study to evaluate the individual and combined effects of Barvar-2 and different doses of P on marigold. Consistent with previous findings, the combined effect of chemical fertilizer and Barvar-2 was found to be statistically insignificant for the measured parameters except for carotenoid content in petals and P uptake in shoot. In addition, the lowest time flowering was obtained when transplant roots and seeds were inoculated with Barvar 2 x 400 mg $P1^{-1}$. Maximum fresh weight, display life, P concentration of shoots and carotenoid content were observed in transplant roots inoculated with bio fertilizer x 400 mg $P1^{-1}$ (Table 3).

Abbasniayzare et al. (2012) carried out a study to compare the effects of bio fertilizers and chemical fertilizers on the growth index of *Spathiphyllum illusion*. Maximum leaf number, dry and fresh leaf weight and spadix size were determined in triple super phosphate + Barvar 2 from these applications. Barvar-2 + Nitrokara had the best effect on leaf size, chlorophyll content and flower stalk height (Table 3).

The production of high quality spike, corm and cormels in gladiolus (*Gladiolus grandiflorus* Ness) is provided by the regular application of chemical fertilizers. Ali et al. (2014) evaluated the effect of "biopower" consisting of *Azospirillum* and *Azotobacter* (free living N_2 fixer), *Rhizobium* (symbiotic N_2 fixer) and P solubilizing bacteria on flower quality and growth of gladiolus grown under greenhouse conditions. With the monoculture of *Azospirillum* longest vase life (11.6 days), higher fresh weight (9.65 g) and better macronutrient uptake percentage (4.76% N, P 0.43% and 3.63% K) were obtained compared to control plants. It also performed better than other treatments in terms of the number of cormel (31.95 cormel plants^{-1}) per plant with the monoculture of *Azospirillum* (Table 3).

In another similar study, Meenakshi et al. (2014) showed that three different bio-fertilizers such as *Azotobacte*r, KSB and PSB and various levels of inorganic fertilizers (applied both alone and in combination) its effect has been evaluated. In the application of 1/2 N, P and K + Azotobacter + PSB + KSB, the maximum number of flowers opened per spike and the available P content in the soil were reported. Maximum fresh and dry spike weight was determined in applications containing 3 / 4th N, P and K + Azotobacter + KSB. Among all applications, the longest vase life was observed in 3 / 4th N, P and K + PSB + STM applications.

Table 3: Examples of PGPR applied in combination with additional treatments on plant growth and yield components of some ornamental plants

Ornamental plant material	Botanical name	Inoculant PGPR	Comments	Biofertilizer	References
Marigold	*Tagetes erecta* L.	*Pseudomonas putida* and *Bacillus lentus*	Plant growth characters, P content in shoot and flower diameter	Barvar-2	Hashemabadi et al., (2012)
Lavender	*Lavandula stoechas* Linnaeus	*Azospirillum brasilense* Sp245	Root growth parameters of cuttings	TwinN	Zulfitri, (2012)
Paese Lily	*Spathiphyllum illusion*	*Pseudomonas putida* and *Bacillus lentus*	The growth index	Barvar-2, Nitrokara	Abbasniayzare et al., (2012)
Gladiolus	*Gladiolus grandiflorus* Ness	*Azotobacter* and *Azospirillum* (free living N2 fixer), Rhizobium (symbiotic N2 fixer) and P solibilizing bacteria	Flower quality and growth of gladiolus	Biopower	Ali et al., (2014)
Marigold	*Tagetes erecta* L.	*Pseudomonas putida* and *Bacillus lentus*	Time flowering, fresh weight, display life, P concentration of shoots and carotenoid content in petals	Barvar-2	Zaredost et al., (2014)
Gladiolus	*Gladiolus grandiflorus* Ness	Potassium solubilizing bacteria (KSB), Azotobacter, phosphate solubilizing bacteria (PSB)	Plant growth and quality of corm and cormels	Inorganic fertilizer	Meenakshi et al., (2014)
Gladiolus	*Gladiolus grandiflorus* Ness	Rhizobium, phosphorus solubilizing bacteria, Azotobacter and Azospirillum	Corms productivity, vegetative and flowering traits, leaf chemical composition and total chlorophyll content	-	Qasim et al., (2014)

Furthermore, a maximum number of corms and cormels per plants were produced in the K + Azotobacter + PSB and 3 / 4th N, P application (Table 3).

The effectiveness of several different PGPR formulations to improve the root growth parameters of cuttings and the development of suitable formulations for PGPR producing IAA have been investigated (Zulfitri, 2012). To further evaluate PGPR's potential in cutting propagation, the highest producer of IAA, *A. brasilense* Sp245, and the most susceptible plant *L. stoechas* (lavender) were selected. Freeze-dried commercial biofertilizer and peat cultures Sp245 resulted in low IAA production. These two treatments were ineffective for *L. stoechas* propagation (Zulfitri, 2012) (Table 3, Figure 5).

Qasim et al. (2014) conducted a trial to evaluate the effect of *Rhizobium,* PSB, *Azotobacter*, *Azospirillum* on the growth and production of gladiolus under field conditions. In general, bacterial cultures significantly improved the flowering and vegetative characteristics, corm yield and chemical composition of gladiolus foliage. Especially *Azospirillum* has been found to have maximum and positive stimulating effect on both reproductive and vegetative characteristics. Significant increases in total chlorophyll content, protein content, total soluble sugar and N, P and K accumulations were observed in the gladiolus plant due to inoculation with *Azospirillum* which was followed by *Azotobacter* treatment (Table 3).

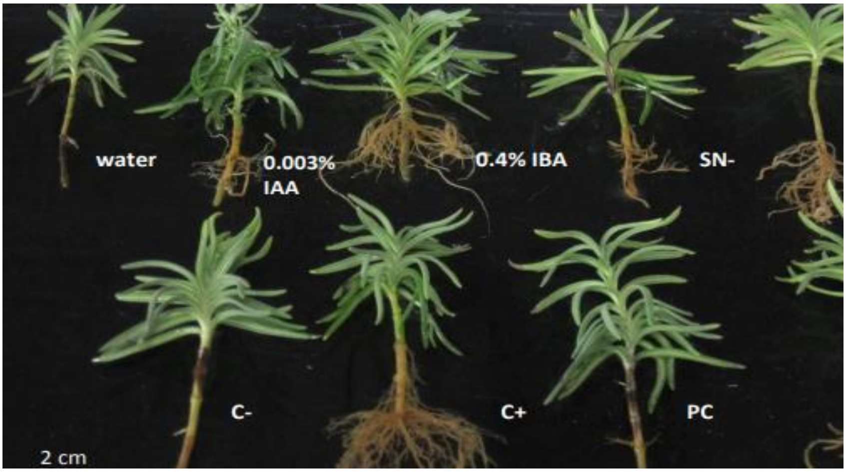

Figure 5: "The difference of root abundance and appearance of *L. stoechas* cuttings at 30 days after immersed with various solutions. SN: supernatant of Sp245, C: culture of Sp245, PC: peat culture of Sp245, CB: commercial biofertilizer. With/without tryptophan (+/-)." (Zulfitri, 2012)

The single and combined effects of selected bio fertilizers such as those prepared from *Azospirillum* and *Azotobacter* on the growth yield and flowering of gladiolus grown in soil treated with different NPK levels was differed (Dalve et al., 2009). However, in the presence of reduced N doses, bio fertilizers significantly affected on the growth, yield and flowering of gladiolus. From the research by Dalve et al. (2009), it was concluded that 25% of N can be saved by applying bio fertilizers in gladiolus cultivation (Table 4).

In studies aiming to reduce the amount of chemical fertilizers used in the production of ornamental plants, both the recommended amount of chemical fertilizers specific to the species and the results of studies on the effects of 50% reduced amount of chemical fertilizers used together with PGPR on ornamental plants are also available. In the study examining the effects of biological fertilizers and NPK fertilizers on the growth characteristics and chemical composition of *Calendula officinalis* L., applications were planned as biological fertilizers (without biological fertilizers, *P. fluorescence* 36, *P. fluorescence* 169, *P. fluorescence* 187, *P. fluorescence* 178, *P. putida* 159) with different NPK fertilizer ratios. With an increase in NPK ratio of up to 100%, a significant increase was seen in all variables when compared to 0 (zero) NPK. The highest concentration of flavonoids were obtained in plants treated with *P. fluorescence* 36 strain. It has been concluded that the use of biological fertilizers or combination with chemical fertilizers has positive effects on the physiological properties of marigold. It is also one of the important results of the research that the use of biological potential fertilizers instead of the continuous use of chemical fertilizers can increase in the sustainability of agriculture and product quality (Arab et al., 2015) (Table 4).

The investigation was conducted to investigate the potential effect of different strains of *Bacillus* and *Pseudomonas* on flowering and growth of chrysanthemum by Kumari et al. (2016). The maximum plant spread and fresh weight of plant were recorded in plants inoculated with BS3 strain of *Bacillus* (SB127) and PS2 strain of *Pseudomonas* (CPA152) in both the years. Maximum number of flowers/plant, fresh and dry weight of flower and flower yield/plant were recorded in plants inoculated with BS3 strains of *Bacillus* (SB127) and PS2 strains of *Pseudomonas* (CPA152) (Table 4).

In another research, the effects on the plant growth of seven different tulip varieties under field conditions, single *P. polymyxa* (BI), *P. putida* (BII), *B. subtilis* (BIII) and *K. cryocrescens* (BIV) isolates (Table 4) and combination of each bacterial isolate with recommended 50% reduced mineral fertilizer) were determined. As a result of the study, the interaction between cultivar and application was found to be important in terms of plant growth parameters of tulips. Applications generally shortened the bulb's exit time in the bulb. Shorter germination time was provided with the combination of MF + BIII (Ekinci et al., 2017) (Table 4) (Figure 6).

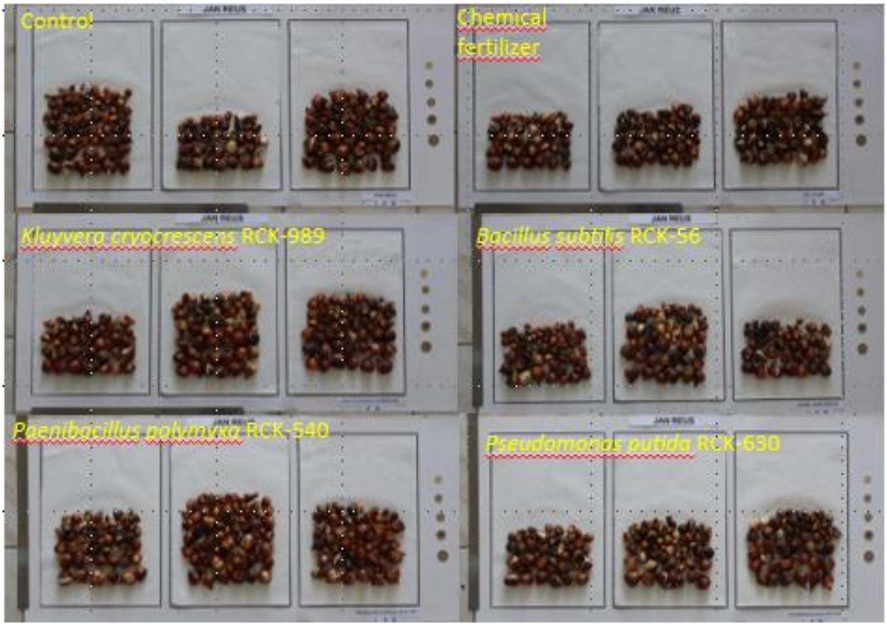

Figure 6: Comparison of chemical fertilizer and control (without chemical fertilizer and bacteria application) applications with applications of different rhizobacteria applied on *Tulipa gesneriana* L. cv. Jan Reus variety (Original by Parlakova Karagöz)

This study was carried out in order to determine the effects of chemical fertilizers, different PGPR formulations and their combinations on some development parameters of poinsettia. Treatments were created as formulation 1, formulation 2, formulation 3, formulation 4, chemical fertilizer (100% RDF), combination of 50% reduced amount of chemical fertilizer with each formulation and control. It has been determined that BIV+CF, BIII+CF, BIV and BII bacteria formulations, which have positive effects on some plant development and quality characteristics of poinsettia, can be used in the poinsettia growing stage. Thus, bacterial formulations may lead to a reduction in the use of chemical fertilizers in poinsettia cultivation (Parlakova Karagöz & Dursun, 2019b) (Table 4) (Figure 7,8).

In a follow up study, Girgin (2019) conducted a similar experiment to determine the effects on the development of the plant by applying chemical fertilizers and rhizobacteria promoting plant growth and their combinations to *Cyclamen persicum*, which has a high commercial value among ornamental plants. The earliest flowering was 75 days in F6K application. The highest plant height, flower stalk, leaf width and tuber length were seen in F4K (*P. polymixa* RK-1981 + *A. xylosoxidans* RK-1982 + *P. putida* RK-1983 + 50% reduced chemical fertilizer) application. It was determined that the application of F5K (*B. brevis* RK-1130 + *B. megaterium* RK-491 + *B. megaterium* RK-716 + 50% reduced chemical fertilizer) increased in the plant crown width the most. The highest values of leaf number, leaf stalk thickness, chlorophyll amount and leaf length was obtained from chemical fertilizer application. The biggest tuber diameter occurred in F1K application. At the end of the research, it was determined that the use of chemical fertilizers can be reduced by using PGPR and rhizobacteria that encourage plant growth have positive effects on the growth and quality parameters of the plant. It has been demonstrated that especially F4K and F6K applications contribute significantly to the development of *Cyclamen persicum* plant (Girgin, 2019) (Table 4).

It was conducted to determine the effects on plant growth characteristics and nutrient content of the growing media of different PGPR formulations, chemical fertilizers and their combinations in two different varieties of poinsettia. The applications were created as the bacteria formulations, the full amount of commonly used chemical fertilizer (150 g·100L^{-1}) (100% CF) and by combining the reduced amount of chemical fertilizer by (75 g 100L^{-1}) 50% with each bacterial formulation and control. In poinsettia cultivation, positive effects of BI and BIII bacterial formulation applications in shortening the time until flowering and early flowering were found. Poinsettia plants soon reached marketable commercial size when treated with BIII+CF treatment compared to control. Poinsettia plants absorbed sufficient nutrients from the growth medium in CF, BI, BI + CF, BII, BII + CF, BIII, BIII + CF applications, and plant growth and biomass were increased. In general, it is concluded from the research results that bacterial formulations can be used for poinsettia production in the grower's greenhouse to reduce the need for chemical fertilizers and improve plant growth (Parlakova Karagöz & Dursun, 2020a).

Potted plants are plants that reduce air pollution indoors, preserve natural leaf and flower aesthetics along their life and sold with pots. In the cultivation of potted ornamental plants, compactness directly affects on the large number of branches and the short distance between internodes. This study was conducted using two varieties poinsettia under greenhouse conditions. The highest mean value for the number of internodes was determined in BI + CF and BII + CF applications. The widest crown width was determined in BII + CF application. Maximum number of side branches, bract diameter and length between internodes were obtained from CF application. The highest mean value for the green leaf area was determined in BI and BIV applications. While the highest average value for green leaf fresh weight was determined in BIV + CF application, the highest leaf fresh weight was found in CF, BII + CF and BIV applications. The maximum leaf area was obtained from BII + CF, BI + CF and CF applications, respectively (Parlakova Karagöz & Dursun, 2019c) (Table 4) (Figure 7).

High amounts of chemical fertilizers and various pesticides are used in poinsettia cultivation. This study was carried out to compare the amount of heavy metals (Zn, Cu, Pb and Cd and B (mg kg^{-1}) metalloid) accumulated when different bacterial formulations and chemical fertilizers were used in poinsettia cultivation. In the study, rooted poinsettia cuttings were used as plant material and the study was carried out under greenhouse conditions. The highest amounts of Zn, Cu, Pb and Cd (mg kg^{-1}) in growth medium were obtained from BIV application. According to CF and control applications, BI+CF application was determined as a decrease of 14.47% and 15.70% in the Zn amount of leaf samples of plants, respectively. It was determined that BI application reduced the amount of Cd of plant leaf samples by 26.58% and 54.69%, respectively, compared to CF and control applications. The highest Pb amount was determined in plant root samples (Parlakova Karagöz & Dursun, 2020b) (Table 4).

Figure 7: The effects on plant growth characteristics of PGPR formulation+ chemical fertilizer combinations in poinsettia (Christmas Feelings cultivar) (Original by Parlakova Karagöz)

In a follow up study, Parlakova Karagöz & Dursun (2020c) conducted a similar experiment to to determine the effects on some color characteristics and nutritional content of bract leaves by applying chemical fertilizers and rhizobacteria promoting plant growth and their combinations to two cultivars of *Euphorbia pulcherrima* Willd ex Klotzsch. The treatments included bacterial formulations. Also, fertilizer treatments included the full amount of commonly used Chemical Fertilizer (CF= 150 g . 100 L^{-1}) and combination of the reduced amount of chemical fertilizer by 50% with each bacterial formulation, and control. In the experiment, the first red leaves, lifetime of the bracts, color characteristics, anthocyanin content, chlorophyll content in green leaves, macro and micronutrient contents of the bracts were evaluated. It was determined that CF and BII+50% CF applications encouraged early coloring of leaves. It was determined that CF, BI + 50% CF and BII + 50% CF applications significantly increased in the chlorophyll content of poinsettia bracts when compared to the control. Darker color of bracts were obtained from BI and BIV applications compared to the control. The highest amount of total N, soluble P, K and Ca have has been found in BII + 50% CF application. It has been determined that bacterial formulations of BI, BIV, BIV + 50% CF and BII + 50% CF have positive effects on some plant aesthetics, quality characteristics and nutritional content of poinsettia and can be used as one of the biological products during the production of poinsettia. Therefore, bacterial formulations can replace or reduce the use of chemical fertilizers in poinsettia production (Table 4).

Figure 8: The effects on root growth characteristics of different PGPR formulations, chemical fertilizer and their combinations in poinsettia (Christmas Feelings cultivar) (Original by Parlakova Karagöz)

CONCLUSION

Until recently, it has been thought that the use of chemical fertilizers, herbicides and pesticides should not be neglected for an agricultural practice to be successful. Initially, these chemicals help the plant to grow, but then show their negative effects. These negativities not only pose a threat to the soil and its inhabitants, but also to human life through the food chain. The soil has become grossly barren and unproductive with the increase in soil pollution, changes in climate conditions, soil-borne pathogen and excessive land use: PGPRs, when used properly, will reduce soil pollution, ecosystem change, and the destruction of soil flora and fauna. A considerable amount of research demonstrating the effect of PGPR on the growth and development of different plants has been reported. However, information on the effect of PGPR on the quality and production of ornamental plants has remained insufficient to date compared to other crops. Therefore, their development as inoculants for the production of ornamental plants continues to be a major challenge. Nevertheless, different PGPR bacteria have been tried with multiple growth-stimulating properties that have a positive effect on the production and quality of flower crops such as marigold, rose, tulip, carnation, poinsettia, aster, chrysanthemum, jasmine, marigold. These beneficial microorganisms improve the uptake of nutrients and improve the quality of ornamental plants by providing other growth-promoting hormones and acting as antagonists, as well as significantly lower cost.

A number of inoculated bacterial strains, often associated with plant rhizosphere under laboratory conditions, have been tested and found to be beneficial for yield, plant growth and crop quality so far. However, the effects of PGPR on field, pot and greenhouse conditions must be known. However, more knowledge and a deeper understanding of their ecophysiology is required for them to be considered and used as successful and potential inoculants. In addition, ornamental plant species should be evaluated separately in terms of the effect of PGPRs. Moreover, there is a need to expand the use of versatile PGPR in floriculture applications.

Table 4: Examples of PGPR applied in combination with additional treatments of **chemical fertilizer** on plant growth and yield components of some ornamental plants

Ornamental plant material	Botanical name	Inoculant PGPR	Comments	References
Gladiolus	*Gladiolus grandiflorus* Ness	Azotobacter and Azospirillum	Growth, flowering and yield	Dalve et al., (2009)
Marigold	*Calendula officinalis* L.	*Psedomonas fluorescence* 36, *P. fluorescence* 187, *P. fluorescence* 169, *P. fluorescence* 178, *Psedomonas putida* 159	The growth characteristics and chemical composition	Arab et al., (2015)
Chrysanthemum	*Chrysanthemum morifolium* Ramat.	Three strains of *Bacillus* (BS1 - SYB101, BS2- SB155 and BS3 - SB127), three strains of *Pseudomonas* (PS1 - WPS73, PS2 - CPA152 and PS3 -P20)	Growth and flowering of chrysanthemum	Kumari et al., (2016)
Tulip	*Tulipa gesneriana* L.	*Paenibacillus polymyxa, Pseudomonas putida, Bacillus subtilis, Kluyvera cryocrescens*	Plant growth and quality of bulb and bulblets	Ekinci et al., (2017)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	*Paenibacillus polymyxa* TV-12E, *Pseudomonas putida* TV-42A, *Pantoea agglomerans* RK-79, *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92, *Bacillus subtilis* TV17C, *Kluyvera cryocrescens* TV-113C, *Bacillus megaterium* TV-6D	Development parameters	Parlakova Karagöz & Dursun, (2019b)
Cyclamen	*Cyclamen persicum*	*Pseudomonas fluorescens* RK-1979, *Bacillus subtilis* RK1977, *Rhodococcus erythropolis* RK-1978, *Bacillus megaterium* RK-504, *Pantoea agglomerans* RK79, *Bacillus subtilis* RK-1984, *Paenibacillus polymixa* RK-540, *Pantoea agglomerans* RK92, *Bacillus pumilus* RK-1980, *Paenibacillus polymixa* RK-1981, *Achromobacter xylosoxidans* RK-1982, *Pseudomonas putida* RK-1983, *Brevibacillus brevis* RK-1130, *Bacillus megaterium* RK491, *Bacillus megaterium* RK-716, *Pseudomonas fluorescens* RK-1105	Development parameters	Girgin, (2019)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	*Paenibacillus polymyxa* TV-12E, *Pseudomonas putida* TV-42A, *Pantoea agglomerans* RK-79, *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92, *Bacillus subtilis* TV17C, *Kluyvera cryocrescens* TV-113C, *Bacillus megaterium* TV-6D	Plant growth characteristics and nutrient content	Parlakova Karagöz & Dursun, (2020a)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	*Paenibacillus polymyxa* TV-12E, *Pseudomonas putida* TV-42A, *Pantoea agglomerans* RK-79, *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92, *Bacillus subtilis* TV17C, *Kluyvera cryocrescens* TV-113C, *Bacillus megaterium* TV-6D	Compactless of plant	Parlakova Karagöz & Dursun, (2019c)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	*Paenibacillus polymyxa* TV-12E, *Pseudomonas putida* TV-42A, *Pantoea agglomerans* RK-79, *Bacillus megaterium* TV-91C, *Pantoea agglomerans* RK-92, *Bacillus subtilis* TV17C, *Kluyvera cryocrescens* TV-113C, *Bacillus megaterium* TV-6D	Heavy metals and B accumulated	Parlakova Karagöz & Dursun, (2020b)
Poinsettia	*Euphorbia pulcherrima* Willd. ex Klotzsch	*Pseudomonas putida* TV-42A, *Paenibacillus polymyxa* TV-12E, *Pantoea agglomerans* RK-79, *Pantoea agglomerans* RK-92, *Bacillus megaterium* TV-91C, *Bacillus subtilis* TV17C, *Bacillus megaterium* TV-6D, *Kluyvera cryocrescens* TV-113C	Some color characteristics and nutritional content of bract leaves	Parlakova Karagöz & Dursun, (2020c)

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CHAPTER 19

INVESTIGATION OF THE FACTORS AFFECTING FIRST FLOWERING TIME OF *TULIPA GESNERIANA* BY PATH ANALYSIS

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INTRODUCTION

Tulipa gesneriana (tulip), which belongs to the Liliaceae family, is an ornamental plant, which has a pear-shaped flower bulb covered with a hard brown shell and is in the group of true bulbous plants. Tulip is a flower that has been loved and adopted enough to be the crown in Turkish culture by naming it a period, and has been a symbol of elegance, delicacy and innocence in the past and present. The tulip have many different flower colors that grow one on a stem (Baytop & Kurnaz, 2003). The development of the tulip plant is completed in as little as a few months. Fertilization is important for the best completion of the development, bulb growth and increase in the number of bulbs. In recent years, the use of biological fertilizers has been encouraged instead of the use of chemical fertilizers, and thus efforts to grow plants with an environmentally friendly approach have been increasing. In addition to bringing the nutrients desired to be given with chemical fertilizers to the plant with biological fertilizers, salt accumulation in the soil due to excessive and unconscious fertilization is prevented. Plant growth promoting bacteria (PGPB) are widely used in agricultural products to improve the growth characteristics of plants and also to reduce the use of chemical inputs (fertilizers and pesticides) that cause environmental damage. In tulip cultivation for cut flower and bulb production, it has been determined that especially in the first stage (green bud period) plant nutrition is important and foliar fertilization is beneficial (Hetman & Laskowska, 1992).

According to Zulfitri (2012), researches on the effect of PGPR applications in the ornamental plants industry are still insufficient. However, in the search for alternative solutions to chemical fertilizers, some researches on ornamental plants and other cultivated plants have shown that biological fertilizers (PGPR) can be successfully applied, thus reducing the dose of chemical fertilizers (Turan et al., 2010; Xu et., 2011; Çakmakçı et al., 2012).

The sale of cut flowers and bulbs on the market contributes to the economy. Flowering time is one of the most important traits with respect to crop yield (Ionescu et al., 2016).

Path analysis is included in the group of alternative multivariate statistical methods within a multivariate structure. Path analysis is an extension of the regression model, where we can analyze not just direct effect of the predictors to the response variable, also the indirect effects of whole predictors to the response variable. The method of path analysis was originated and developed by Sewall Wright in the 1920s. Deducing genetic consequences of a continued inbreeding system was Wright's most accomplished use of path analysis method (Wright, 1921a,b; 1934; 1968; Bondari, 1990). This method makes a division between the direct, indirect and total effects of one variable on another. The direct effect appears to be the impact of one variable on another variable. This variable is not conciliated by any other variable, which comes to be the part of the model. The effect of one variable on another that is intervened by, or infiltrates, minimum one other variable in the system is the indirect effect. The sum of the direct and indirect effects is called the total effect (Everitt & Dunn, 1991). The method of path coefficients for a long time has been a simple, to a great extent productive method for genetic and environmental variables analysis in outward formed breeding-population systems that is closed (Li, 1975). Animal and plant breeders have widely used this method (Bondari, 1990; Boydak et al., 2002; Karayel & Bozoğlu, 2009; Aytekin et al., 2016).

In this study, leaf number, leaf length, flower length, flower diameter, stalk thickness and leaf diameter parameters were used to calculate direct and indirect effects to the first flowering time of *Tulipa gesneriana*.

MATERIAL AND METHOD

*Tulipa gesneriana "*Lady Van Eijk" tulip bulbs (Figure 1) purchased from "Asya Lale" company were used as plant material in the study.

Figure 1: *Tulipa gesneriana* "Lady Van Eijk" (Original by Çığ)

The applied bacterial isolates are *Brevibacillus choshinensis* (TV53D), which was isolated from the Lake Van Basin and had been carrying both nitrogen-fixing and phosphate solibilizing properties, which were previously diagnosed with the MIS system and demonstrated in the greenhouse and field conditions for Plant Growth Promoting Bacteria (PGPB) (Location: Çakırbey Village / Van) and nitrogen-fiixing *Pseudoalteromomas tetraodonis* (TV126C) (Location: Ulupamir Village / Van) were selected from the species and were obtained from Siirt University Faculty of Agriculture Field Crops Department in stock.

Method

The study was established in Siirt University Faculty of Agriculture Application Garden in December 2019.

Table 1: Application methods

No	Application	MIS diagnosis result	Feature	Application time
1	Control	Bulbs without bacteria treatment	-	-
2	Bacteria 1	*Brevibacillus choshinensis* (TV53D)	Nitrogen fixing and phosphate solubilizing together	Bacteria application only to the bulb
3	Bacteria 1	*Brevibacillus choshinensis* (TV53D)	Nitrogen fixing and phosphate solubilizing together	Spray application only to the plant
4	Bacteria 1	*Brevibacillus choshinensis* (TV53D)	Nitrogen fixing and phosphate solubilizing together	Bacteria application to bulb + spray application to plant
5	Bacteria 2	*Pseudoalteromomas tetraodonis* (TV126C)	N fixing	Bacteria application only to the bulb
6	Bacteria 2	*Pseudoalteromomas tetraodonis* (TV126C)	N fixing	Spray application only to the plant
7	Bacteria 2	*Pseudoalteromomas tetraodonis* (TV126C)	N fixing	Bacteria application to bulb + spray application to plant

As seen in Table 1, a total of 7 treatments were applied to tulip bulbs, including the control group. Bacteria were applied to tulips in three different ways.

The effects of bacterial treatments on the parameters shown below were examined:

First Flowering Time (days): The time when the tulip flower opens, is calculated from planting.

Number of leaves (pieces): The total number of leaves on the plant has been determined.

Leaf length (mm): The average length of leaves on the plant was determined by digital caliper.

Leaf width (mm): The average width of the leaves on the plant was determined by digital caliper.

Plant height (mm): The distance from the soil surface of the plant to the top of the flower petals was measured with a digital caliper.

Flower length (mm): The distance between the junction of the flower with the stem and the top of the petals was measured with a digital caliper.

Flower width (mm): The distance between the petals opposite each other was measured with a digital caliper.

Stem thickness (mm): Flower stem thickness was measured from the bottom with a digital caliper.

Additionally, indirect effects are vital in a path model as much as direct effects (Alwin & Hauser, 1975). The indirect effect between variables is defined as a continuous paths line that can then be drawn continuously from the first to the second through other variables. This kind of indirect effect is measured by multiplying the direct effects corresponding to the mentioned paths (Arminian et al., 2008).

The basic components of path analysis are the path diagram and the estimation of path coefficients. Path diagrams are most useful for the analysis of two or more equations. This time we are talking about two or more equations that connect observed or hidden variables and residual terms in a system. This diagram is generally easy to interpret. Here, when only the equations of a model are examined, relationships that may be out of attention are easily revealed.

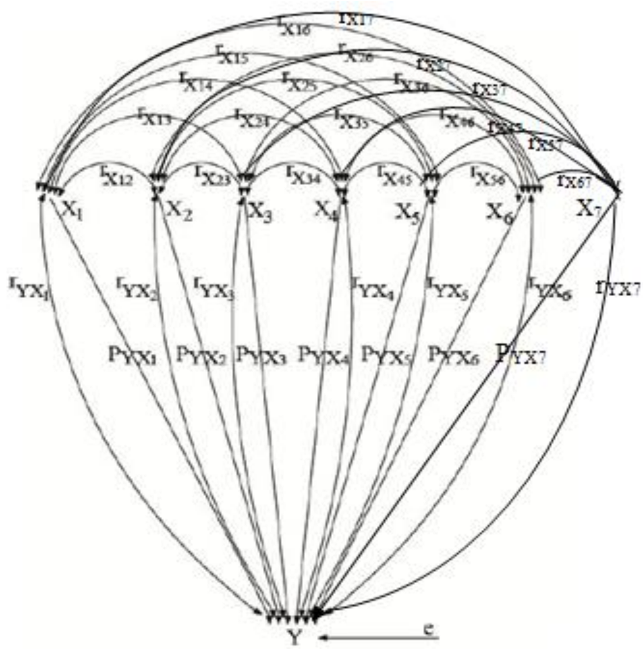

Figure 2: Path diagram for FFT prediction model

In Figure 2, Y is the First Flowering Time (FFT), X_1 is Leaf Number (LF), X_2 is Leaf Length (LL), X_3 is Plant Length (PL), X_4 is Flower Length (FL), X_5 is Flower Diameter (FD), X_6 is Stalk Thickness (ST) and X_7 is Leaf Diameter (LD). The direct effects of X_i variables (i=1, …, 7) on Y feature were indicated with P_{YXi}. Indirect effects of X_i predictor variables to Y were indicated with $r_{ij}P_{YX_i}$ (i,j=1, … 7).

In the path analysis, indirect effect of independent variables plays an important role on the response variable (Ulukan et al., 2003). MINITAB® Release 14.12.0 was used for analysis.

Correlation coefficients among the FFT and other morphological features should be divided to the sum of direct and indirect effects as shown below:

$$r_{YX_1} = P_{YX_1} + r_{12}P_{YX_2} + r_{13}P_{YX_3} + \cdots + r_{17}P_{YX_7}$$
$$r_{YX_2} = P_{YX_2} + r_{12}P_{YX_1} + r_{23}P_{YX_3} + \cdots + r_{27}P_{YX_7}$$
$$\vdots$$
$$r_{YX_7} = P_{YX_7} + r_{17}P_{YX_1} + r_{27}P_{YX_2} + \cdots + r_{67}P_{YX_6}$$

RESULTS AND DISCUSSION

Descriptive statistics concerning all parameters were given in Table 2. As can be seen from the descriptive statistics, the first flowering time of *Tulipa gesneriana* was observed as the shortest 79 days and the longest 92 days. The average FFT was calculated as 85.26 days.

Table 2: Descriptive statistics of all morphological parameters

Parameter	N	Mean	Std. Deviation	Minimum	Maximum
First Flowering Time (day) (FFT)	27	85.26	3.024	79	92.33
Leaf Number (LN)	27	3.06	0.16	3	3.66
Leaf Length (mm) (LL)	27	78.40	7.15	61.63	86.69
Plant Length (mm) (PL)	27	205.54	38.93	103.71	254.59
Flower Length (mm) (FWL)	27	66.09	8.47	50.46	77.56
Flower Diameter (mm) (FWD)	27	60.17	8.38	32.01	69.52
Stalk Thickness (mm) (ST)	27	6.31	0.78	4.35	8.68
Leaf Diameter (mm) (LD)	27	37.74	5.04	28.06	50.69

Correlation coefficients between all parameters were given in Table 3. When we examined the correlation coefficients, statistically significant relationships with FFT were found between LN ($p < 0.05$) and FL ($p < 0.01$). That is, the increase in the number of LNs causes a shortening of the FFT time. The increase in FL also causes an increase in the FFT time. Although it is not statistically significant, in addition to LN, ST and LD properties also have a decreasing effect on FFT. In other words, the increase in these features enables the FFT time to be shortened. Other features have an increasing oriented relationship with FFT. It was found a positive and statistically significant ($p < 0.05$) relationship between FFT and LL on different hyacinth varieties in Mikail et al. (2019) study. The correlation coefficients of FD, FL and FD with FFT were found negative and statistically significant. The correlation coefficient between ST and FFT was found non-significant, that makes it similar to our work.

Table 3: Correlation matrix between parameters

Parameters	LN	LL	PL	FL	FD	ST	LD
LL	-0.167						
PL	-0.129	0.679**					
FL	-0.361	0.643**	0.851**				
FD	-0.223	0.479*	0.725**	0.711**			
ST	0.201	0.272	0.523**	0.257	0.484*		
LD	-0.183	0.702**	0.452*	0.288	0.365	0.266	
FFT	-0.463*	0.096	0.209	0.525**	0.234	-0.170	-0.109

*:p<0.05; **:p<0.01 (LN-Leaf Number, LL-Leaf Length, PL-Plant Length, FL-Flower Length, FD-Flower Diameter, ST-Stalk Thickness, LD-Leaf Diameter, FFT-First Flowering Time)

After the correlation coefficients were calculated, the direct and indirect effects of the seven properties examined by applying path analysis and their percentage effect shares in their correlation coefficient were determined. Path analysis results were illustrated in Table 4. According to the multiple linear regression analysis results the prediction model can be written as follows:

FFT=1.1762FL-0.1686LN-0.2541LL-0.0059LD-0.5461PL-0.1049FD-0.0309ST

As can be seen from Table 4, although the LN feature has a statistically significant ($p < 0.05$) effect on FFT, when this effect is broken down to direct and indirect effects, only 22.9% of this effect is directly between LN and FFT. The indirect effect share of LN over FL has the highest (57.7%) share in the correlation coefficient. In the study conducted by Mikail et al. (2019) on different hyacinth varieties, the direct effect of LN on FFT was also found to be statistically insignificant. Likewise, the direct effect of LD on FFT was found to be insignificant. The direct effect of the FL feature on the FFT was found to be statistically significant ($p < 0.05$) with a percentage of 60.3%. The indirect effect of FL over PL, on the other hand, in addition to being opposite, has a percentage of 23.8%.

Table 4: Direct and indirect effects of investigated traits to the First Flowering Time (FFT)

Parameters	Direct effects	Indirect effects							Total effect
		LN	LL	LD	PL	FL	FD	ST	
LN	-0.1686	-	0.0423	0.0010	0.0706	-0.4251	0.0233	-0.0062	-0.463*
%	22.9		5.7	0.1	9.6	57.7	3.2	0.8	100.0
LL	-0.2541	0.0281	-	-0.0041	-0.3709	0.7557	-0.0501	-0.0084	0.096
%	17.3	1.9		0.3	25.2	51.4	3.4	0.6	100.0
LD	-0.0059	0.0308	-0.1784	-	-0.2470	0.3383	-0.0383	-0.0082	-0.109
%	0.7	3.6	21.1		29.2	39.9	4.5	1.0	100.0
PL	-0.5461	0.0218	-0.1725	-0.0026	-	1.0010	-0.0760	-0.0162	0.209
%	29.7	1.2	9.4	0.1		54.5	4.1	0.9	100.0
FL	1.1762*	0.0609	-0.1632	-0.0017	-0.4647	-	-0.0745	-0.0079	0.525**
%	60.3	3.1	8.4	0.1	23.8		3.8	0.4	100.0
FD	-0.1049	0.0375	-0.1215	-0.0021	-0.3959	0.8360	-	-0.0150	0.234
%	6.9	2.5	8.0	0.1	26.2	55.3		1.0	100.0
ST	-0.0309	-0.0338	-0.0690	-0.0015	-0.2858	0.3019	-0.0508	-	-0.170
%	4.0	4.4	8.9	0.2	36.9	39.0	6.6		100.0

*:$p<0.05$; $R^2=0.538$ (LN-Leaf Number, LL-Leaf Length, PL-Plant Length, FL-Flower Length, FD-Flower Diameter, ST-Stalk Thickness, LD-Leaf Diameter, FFT-First Flowering Time)

CONCLUSIONS

Correlation coefficients have shown that first flowering time of Tulip was positively and significantly correlated with flower length and negatively and significantly correlated with leaf number. The path analysis has shown that just flower length had the positive significant direct effect to the first flowering time. These parameters may be useful as predictors of the best first flowering time.

Path analysis has an important role in revealing the relationships between variables in multivariate analysis and calculating their direct and indirect relation shares with the predicted variable. In agricultural sciences, the direction and amount of the relationships between the characteristics studied in natural phenomena, as well as the calculation of the impact shares in the total relationship are very important in future predictions or finding the best yield. For this reason, estimation of first flowering time, which is an important feature in ornamental plant cultivation, or determining the factors affecting its shortening, will ensure that this feature is kept under control by the cultivator.

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CHAPTER 20

OUTLOOK ON ORNAMENTAL PLANTS SECTOR IN TURKEY

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INTRODUCTION

Ornamental plants sector has been playing increasingly important role in the agricultural sector. Ornamental plants are economically and visually valuable. The economic importance of ornamentals has been increasing in many countries, and international demand has rapidly expanded. Cut flowers represent the largest segment of the industry followed by flowering pot plants, tree and nursery crops, flower bulbs, and other propagation material (Lawson, 1996).

Ornamental plants provide better income from a unit area with higher profitability. There is huge untapped flower production potential in our country which could benefit a large segments of the weaker sections of the society. Both the domestic market and the export potential of flowers and ornamentals are tremendous. Besides, earning foreign exchange and improving the national income, the floriculture business being labour intensive generates gainful employment to rural youth. (De, 2017).

Mature domestic producers are North East Asia (China and Japan), North America and Europe. Mature export producers are Colombia, Ecuador and Kenya. Advantages of natural resources, location, altitude, labour force and other economics have propelled the growth of volume and value of cut-flowers grown in these countries (Figure 1). Latin America and Africa production is increasing very rapidly. Ornamental plants produced for domestic markets in major producers such as India, China, USA, Japan, Mexico, Brazil, Thailand, etc. But some producers such as Ecuador and Colombia produced it for export (AIPH, 2019).

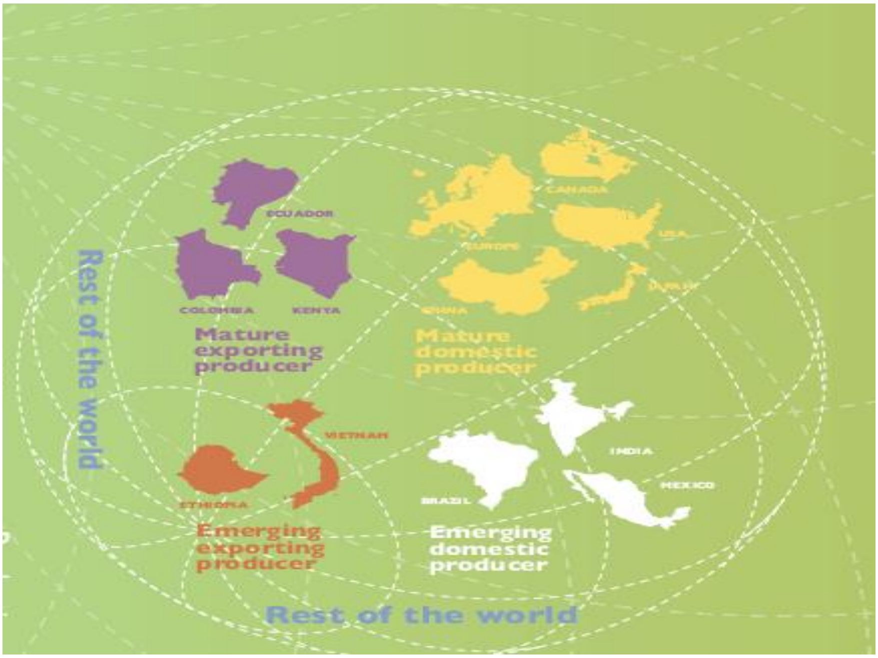

Figure 1: Domestic producer and exporting producers in world (AIPH, 2019)

The production area of ornamental plants in the world for 2017, at 1.78 million ha, is up 17.75% from 2009. Out of all the continents, Asia is the largest, with an area of 1.30 million ha (%72.92). North America ranks second, with a land area of 209.46 thousand ha. Outdoor ornemantal plants, at 1.11 million ha in 2017, is the largest group according to production area, up 42.60% from 2009. Cut flowers and indoor ornamental plants are the 2nd largest group, at 650 thousand ha in 2017 and down 7.46% from 2009. In 2017, the total production value was €65.21 billion. Two leading production continets are Asia-Pasific, €29.23 billion and Europe, €22.08 billion (Kazaz et al, 2020).

In export market, the Netherlands is still a major conflux in global flower trade, but the four cut flower exporters close to the equator (Colombia, Kenya, Ecuador and Ethiopia) are gathering speed. The Netherlands plays an important role in the global cut flowers trading as it has more than 40% export share. The main importers are Europe, USA and Japan. The worldwide market for flower and ornamental plants is expected to grow at a compound annual growth rate of roughly 6.3% over the next five years, will reach 57400 million US$ in 2024, from 42400 million US$ in 2019 (Anonymous, 2019).

Turkey is besides being an rich country with regard to genetic diversity and endemism because of its geographical structure difference. Due to this conditions, many ornamental plants are cultivated or gathered from the natural vegetation. Ornamental plant production in Turkey started during 1940's in Istanbul with cut flower production and its surroundings and then spread to other provinces (Titiz et al., 2000).

After 1985, there has been an expeditious improvment in the ornamental plant sector due to promoting export oriented production and facilities for importing replica material. Revenue increased as a result of the increase in ornamental plant exports due to becoming different in the apprehension of the growers (Çelik & Arisoy, 2013). A wide variety of imported flower pots in the domestic market cut flower sales significantly reduced. This situation continued until 1990. Cut flower production has revitalized after 1990 with export to Bulgaria, Romania and Russia (Yazgan et al., 2005).

1. ORNAMENTAL PLANT PRODUCTION IN TURKEY

Ornamental plants can be divided four groups: cut flowers, outdoor and indoor ornamental plants, and natural flower bulbs (Zencirkiran & Gürbüz, 2009). Outdoor ornamental express the plant species used in outdoor landscape applications for aesthetic and functional purposes (Güneş et al., 2019). Indoor ornamental plants are plants used to create a green space in homes, offices and halls. Cut flowers are flowers and flower buds that have been cut from the plant bearing it suitable for bouquets, wreaths, corsage and special flower arrangements (Anonymous, 2001).

In 2019, ornamental plants production area were 5247.74 ha in Turkey. Of this area, 71.84% represents outdoor ornamental plants, 23.58% cut flowers, 3.80% indoor ornamental plants and 0.78% natural flower bulbs (Figure 2).

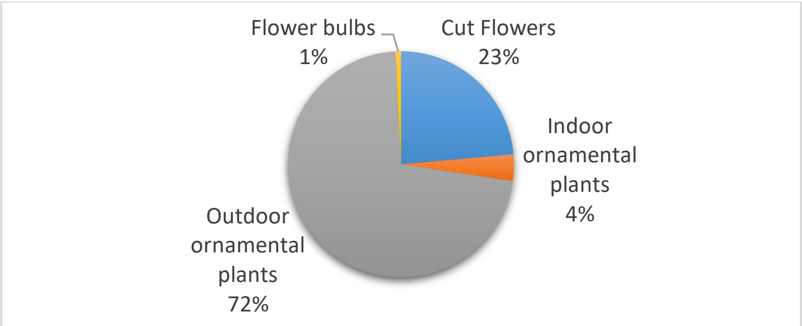

Figure 2: Ornamental plant production areas in Turkey in 2019 (TUIK, 2019)

The production area of ornamental plants, which had been 4636.70 ha in 2010, increased by 13.18% and reached to 5247.74 ha in 2019. Indoor ornamental plants,cut flowers and outdoor ornamental plants production areas increased while natural flower bulbs production area decreased from 2010 to 2019 in Turkey. The increase rates in indoor ornamental plants, cut flowers and outdoor ornamental plants were 99.60%, 12.77% and 11.36%, respectively. On the other hand, production area of flower bulbs showed a decrease of 24.10% (Figure 3).

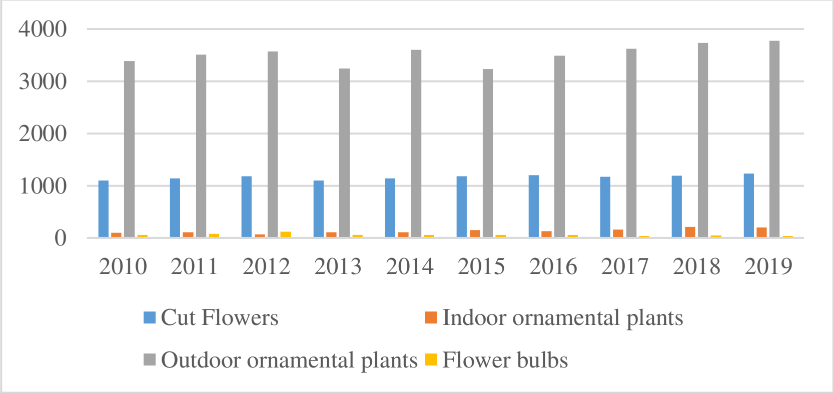

Figure 3: Ornamental plant production areas in Turkey according to years (ha) (TUIK, 2019; SUSBIR, 2019)*

*The tulip bulb production area in Konya province (40 ha) has been taken into consideration for outdoor ornamental plants

In 2018, the provinces with the highest production are Izmir (31.37%), Sakarya (20.86%), Antalya (11.50%), Yalova (6.87%), Bursa (5.77%) and Istanbul (1.07%). The most significant increase was in Izmir. The production area of ornamental plants in Izmir, which had been 780.30

ha in 2009, increased by 108.27% and reached to 1625.10 ha in 2018 (Figure 4).

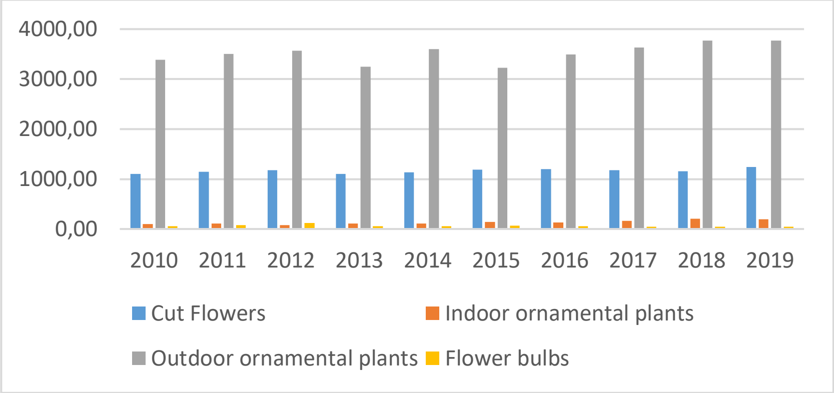

Figure 4: Ornamental plant production areas in Turkey according to provinces (ha) (2009-2018) (SUSBIR, 2019)

In 2019, the production of ornamental plants was 1443.52 million and increased 19.02% compared to 2013. Ornamental plants production consists of cut flowers at 63.64%, outdoor ornamental plants at 29.72%, indoor ornamental plants at 3.01% and natural flower bulbs at 3.64% (Figure 5). The top three cut flowers are carnation, gerber daisy, rose (cut) (Figure 6).

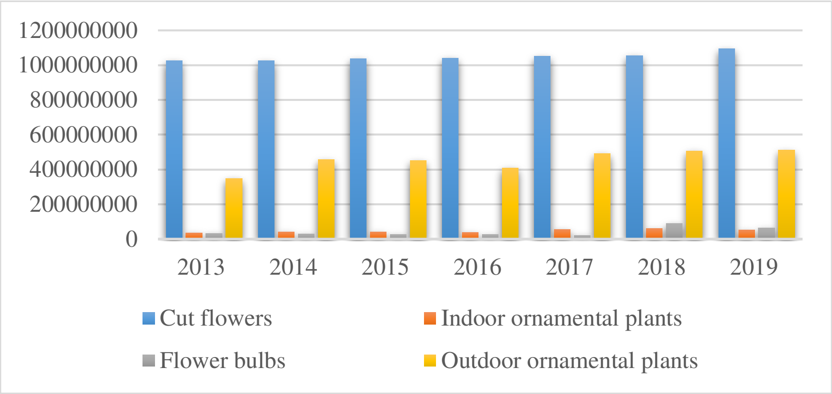

Figure 5: Ornamental plant production in Turkey (TUIK, 2019)

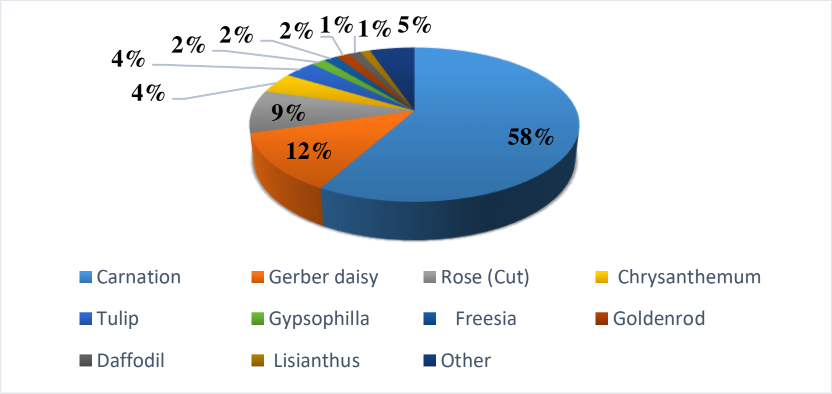

Figure 6: Production of cut flowers (TUIK, 2019)

Ornamental plants are produced in open areas and in greenhouses. While greenhouse ornamental production in Turkey were 1140.81 million in 2013, it's increased by 8.60% in 2019 and reached 1238.97 million. Cut flower production (81%) took the first place. This is followed by outdoor ornamental plants (15.52%), indoor ornamental plants (3.33%), flower bulbs (0.15%), respectively (Figure 7). The top three cut flowers in greenhouses are carnation, gerber daisy, rose (cut).

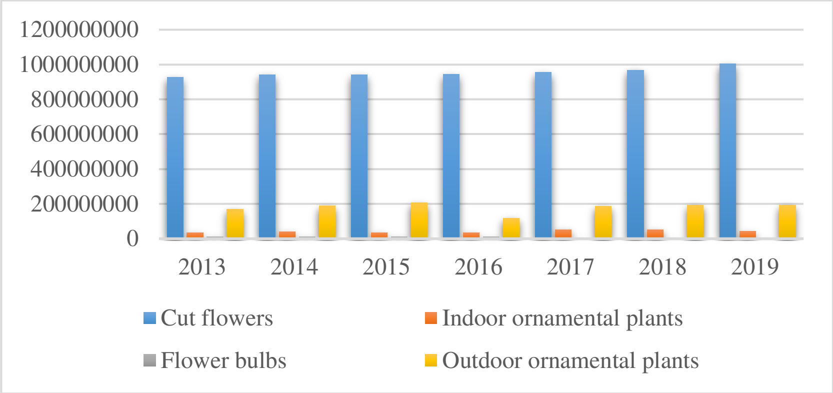

Figure 7: Greenhouse ornamental plant production in Turkey (TUIK, 2019)

2. MARKETING

Ornamental plants sector poses a significant potential in Turkey due to Turkey's suitable climatic and geographical conditions, rich biodiversity, proximity to markets, cheap labor and alternative renewable energy sources (Kazaz et.al., 2015). The sector consists of mostly cooperatives formed by the producers who produce for the domestic market and private sector export organizations (Sayın & Sayın, 2004).

Marketing is a very important in the ornamental plants sector. Cooperative auctions, have been an important tool floriculture product sales. A floriculture production chain starts in general with the grower and reaches the domestic consumer as it is sold by private auction, cooperative auction, wholesaler and the retailer (Figure 8). Nowadays, online sales is soaring but flowers and plants are still mainly bought through traditional sales channels such as florists.

Kazaz et al. (2020) stated that, flower and grass seeds should be included in data and some data in the live plants group should not be included because they are not considered as ornamental plants. It was necesarry to evaluate foreign trade data of ornamental plants in a healthy way. In this study, the data were prepared in this direction.

The value of Turkey's import and export are increasing. In the period from 2010 to 2019, the total value of export , increased by 89.19% and reached to $108.67 million in 2019. The total 2019 ornamental plants exports increased by +8.38% when compared to 2018.

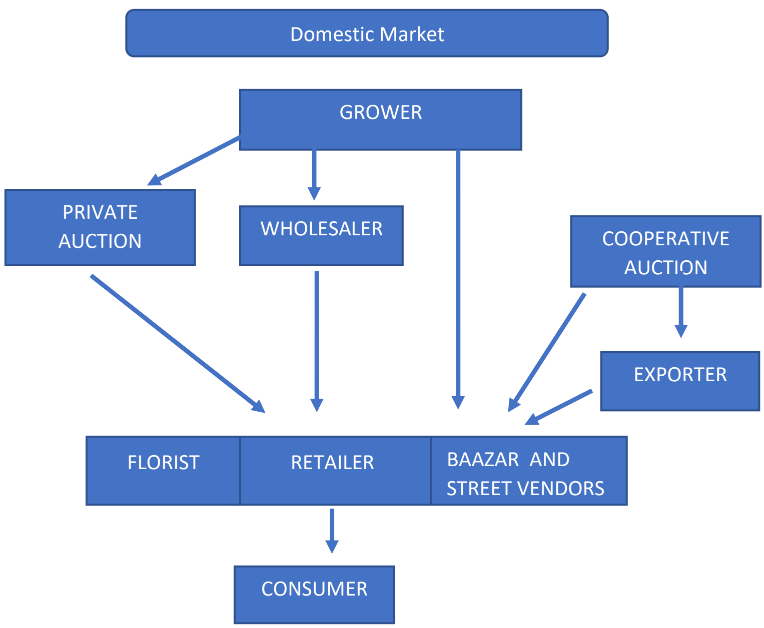

Figure 8: The marketig chain of ornamental products in domestic market (Subaşı & Yeler, 2012)

Last year the export value increased by +15.94% compared to 2017. Of ornamental plants exports' amount in 2019, live plants accounted for about 57.67%, cut flowers for 33.00%, foliage for 7.55%, flower sedds for 3.52% and flower bulbs for 1.28%. (Figure 9). The main importers of Turkey are the Netherlands followed by Germany, Uzbekistan, United Kingdom, Azerbaijan, Turkmenistan, Iraq, Georgia, Bulgaria, USA and Romania. In addition, geophytes harvested from the flora of Turkey are being sold primarily to the Netherlands and other European countries such as Denmark, Switzerland, Germany, Italy, Great Britain, Bulgaria, and France, as well as to the USA and Japan (Zencirkıran & Gürbüz, 2009).

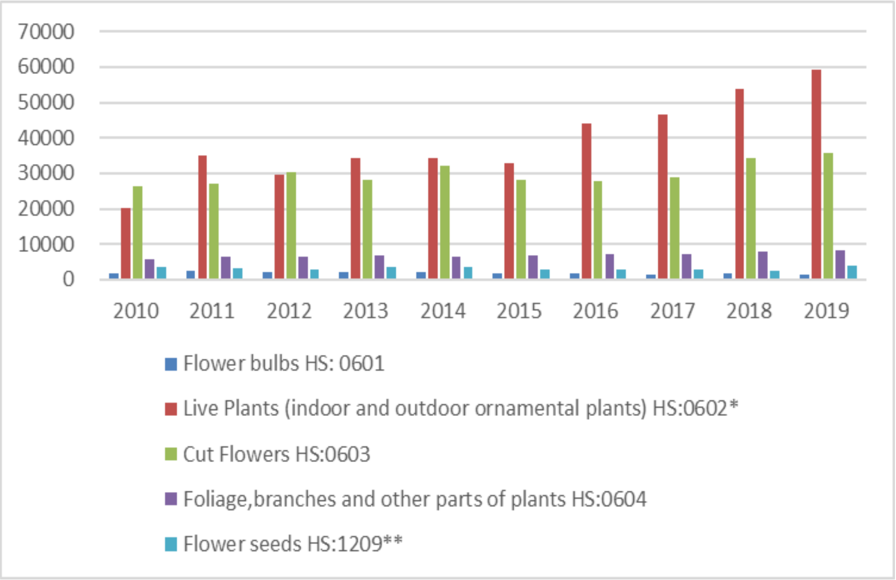

Figure 9: Export value of ornamental plants in Turkey (US Dollar thousand) (Trademap, 2019)

*060220900019,060220100000,060290100000,060290300000 codes have been removed
**120929800018, 120930000000, 120999910000 codes have been added

Besides domestic production, Turkey also imports many ornamental plants from other countries, primarily Netherlands, Italy and France. The value of imports of ornamental plants decreased 0.60% from $44.36 million to $44.10 millions from 2010 to 2019. In 2018 the import value $62.23 millions. The number shrank last year by -29.13% to $44.10 million. The value at import of ornamnetal plants are on a declining trend (Figure 10). Roses imports are the largest component of the Turkey's flora trade, followed orchids, chrysanthemums.

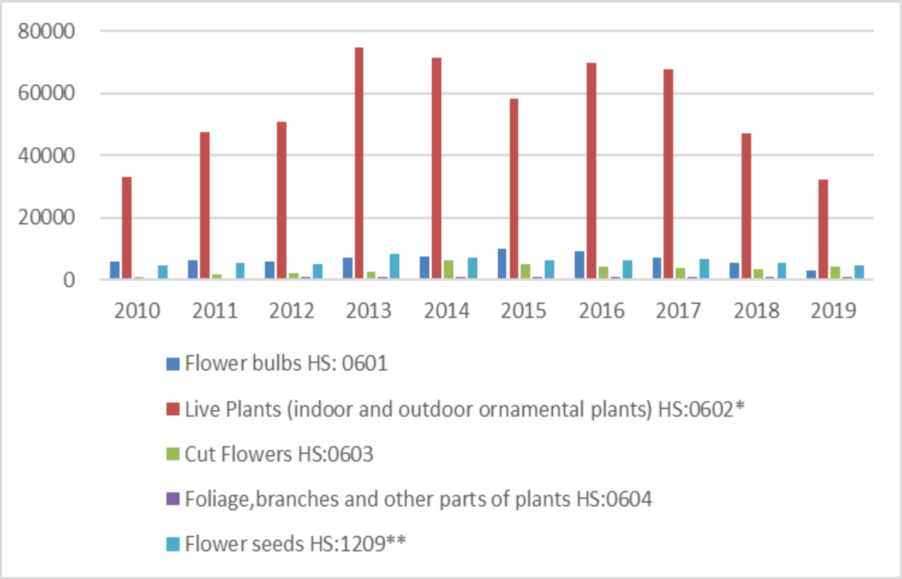

Figure 10: Import value of ornamental plants in Turkey (US Dollar thousand) (Trademap, 2019)

*060220900019,060220100000,060290100000,060290300000 codes have been removed
**120929800018, 120930000000, 120999910000 codes have been added

3. PROBLEMS AND SOLUTIONS

In the ornamental plants sector, there has been a significant increase in production areas and exports, product variety has increased and there have been developments in search for different markets. Growers also are starting to diversify their crops and varieties and the technology level is increasing. However, when the researches and reports made from the past to the present are examined, it is determined that many problems in the ornamental plants sector still continue. The problems in the ornamental plants sector of Turkey are: foreign dependency for materials, excessive imports, small size farms, lack of technology usage, unrecorded production, inequality of value added tax, inadequacy of R&D and breeding studies, lack of expert and technical staff, recording of data and growers (Onay, 2008; Ay, 2009; Karagüzel, et al., 2010; Kazaz et al., 2015; Kızılkan, 2016; Anonymous, 2020a, Anonymous, 2020b).

The following recommendations have been developed in order to enable the ornamental plant sector to participate more effectively in the national economy;

- Foreign dependency in production material causes increase in production costs and negatively affects the international competitiveness. Cultural methods should be practiced and natural and endemic plant species should be taken into culture in order to reduce foreign dependency. Domestic, high quality and high yield ornamental plant species and varieties should be developed. For this purpose, R&D studies should be carried out in cooperation with universities, public institutions and private sector. In addition, breeding studies should be increased. At the same time, making production planning and product specialization of growers is important for the development of the sector (Sayın & Sayın, 2004; Karagüzel, et al., 2010; Kazaz et al., 2015; Kızılkan, 2016).

- Carnation sharing 95% of the Turkey total export value. This situation, which restricts export, should be eliminated by providing product variety for export (Kazaz et al., 2020).
- Excessive imports in the ornamental plants sector hinders the development of the sector. Local governments should be encouraged to use domestic species in landscaping. In addition, government lands can be rented for the long-term production of ornamental plants (Karagüzel, et al., 2010; Kazaz et al., 2015; Cebeci, 2019).
- Value added tax differences should be eliminated and the value added tax rate should be reduced to 8% in order to alleviate the tax burden on growers (Kazaz et al., 2015; Kızılkan, 2016; Anonymous, 2020a).
- Internal consumption is very low. Consumers should be made aware of ornamental plants by advertising and promotional activities. In addition, consumers' preferences should be taken into account in production. Furthermore, training centers for diploma course for training the personnel in floriculture should be set up (Aydınşakir et al., 2014).
- Issues such as harvesting, classification, packaging and transportation are not taken into consideration. For this reason, both

growers and intermediaries should be made aware of this issue (Örük & Engindeniz, 2019).

- Marketing infrastructure (cold storage, processing and packing houses, etc.) facilities should be improved and their number should be increased (Örük & Engindeniz, 2019).
- Modern and large greenhouses should be built and modernization of existing greenhouses provided (Örük & Engindeniz, 2019). Using renewable energy resources (geothermal, wind, solar) in greenhouses will reduce input costs.
- Raising the awareness of growers about the use of chemical fertilizers and pesticides will reduce both input costs and the damage to the environment. Furthermore soilless agriculture, good agricultural practices and organic farming methods should be widespreaded (Örük & Engindeniz, 2019).
- Ensuring product standardization is very important in terms of quality and efficiency. Ornamental Plants Quality Standards should be created for all product groups (Kazaz et al., 2020). Imposing obligations in the implementation and supervision of quality standards prepared for ornamental plants will provide significant contributions to the sector
- Growers should first research existing and potential markets and direct their production in accordance with the demands of these markets. Therefore, market research should be done before production (Örük & Engindeniz, 2019).

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