CURRENT RESEARCH IN AGRICULTURE, FORESTRY AND AQUACULTURE

JUNE 2022

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gece kitaplığı

İmtiyaz Sahibi / Publisher • Yaşar Hız

Genel Yayın Yönetmeni / Editor in Chief • Eda Altunel

Kapak & İç Tasarım / Cover & Interior Design • Gece Kitaplığı

Editörler / Editors • Prof. Dr. Taner Akar
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Birinci Basım / First Edition • © Haziran 2022

ISBN • 978-625-430-204-6

Gece Kitaplığı / Gece Publishing
Türkiye Adres / Turkey Address: Kızılay Mah. Fevzi Çakmak 1. Sokak
Ümit Apt. No: 22/A Çankaya / Ankara / TR
Telefon / Phone: +90 312 384 80 40
web: www.gecekitapligi.com
e-mail: gecekitapligi@gmail.com

Baskı & Cilt / Printing & Volume
Sertifika / Certificate No: 47083

Current Research in Agriculture, Forestry and Aquaculture

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CONTENTS

CHAPTER 1

CHRONIC EFFECTS OF CARBAMAZEPINE ON REPRODUCTION AND PHYSIOLOGICAL TRAITS OF DAPHNIA MAGNA

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Introduction

Pharmaceuticals in the aquatic environment as unmetabolized compounds or as purely metabolites exhibit high biological activity and have adverse effects on aquatic life even at low concentrations (Oetken et al., 2005). Their residues are not completely removed during their passage through the wastewater treatment plants and they can be detected in surface waters globally (Oldenkamp et al., 2019).

Carbamazepine is a compound resistant to environmental degradation and is considered one of the most durable pharmaceutical compounds in the aquatic environment (Ternes et al., 2003). As a pharmaceutical compound it can enter the environment in several ways such as inappropriate treatment of hospital wastewater, unused or semi-consumed drugs in dishwashers, toilets, trash cans, drainage of unabsorbed livestock drugs through the urine, stool of animals in the farms and pastures and unauthorized disposal of expired medicines (Darabi et al., 2019). Tegretol (commercial form of carbamazepine) is a drug with a great deal of worldwide consumption. Carbamazepine concentrations are often reported in the range of 1 to 3600 ng/l in hospitals, surface water, wastewater of the treatment plants and from 10 to 443 mg/l in rivers receiving wastewater treatment effluents (Gadipelly et al., 2014; Munoz et al., 2009).

Daphnia magna is the preferred micro-organisms in laboratory experiments because of its rapid reproduction and easy observation under the microscope. It is abundant in freshwater and has been shown as one of the most aquatic organisms susceptible to effects of the pharmaceutical exposure (Kim et al., 2007). On the other hand, the *Daphnia magna* represents an excellent type of invertebrate model for studying aquatic environments therefore it can be used in the determination of the effect of emerging pollutants at different biological levels (Rivetti et al., 2016).

This paper investigates the effects of human pharmaceuticals on freshwater invertebrates in the laboratory by examining the influence of the three concentrations of carbamazepine (Tegretol) (1,25, 1,75 and 2,25 mg/L) on the heartbeats, fertility, molting and mortality figures on *Daphnia magna*.

Material and Methods

Experimental animals and environmental conditions

This research was carried out in the Fisheries and Diseases Laboratory of the Faculty of Veterinary Medicine at Tekirdağ Namık Kemal University where *Daphnia magna* has been cultured since 2020. The daphnids used in the study were filtered with the help of a fine-mesh strainer to obtain standard size organisms and kept at 24-25^{0}C water temperature. The experiment was conducted in a completely randomized design with 4 treatments and 3 replications. A total of 240 gynandromorphic daphnids were randomly selected, sorted equally into control, 1,25, 1,75 and 2,25 mg/L groups. They were placed into 12 air-only containers filled with 4 liters freshwater each consisting of 20 Daphnia and acclimatized for one week. A single *D. magna* clone produced in the laboratory was used for all assays. Daphnia was fed daily with Daphnia growth food (Inve O.range Start-S 100-200μ) with an ingredient of 56% min raw protein, 13% raw fat, 1% raw cellulose, 1,2% max Ca, 1,3 max P and 10% ash. The culture medium was changed every other day and neonates were removed within 24 h. Photoperiod was set to 16 h light: 8 h dark cycle and temperature at 24±1°C. During the 42-day trial period, concentrations of Carbamazepine Oral Suspension ranging from 1,25, 1,75 and 2,25 5 mg/L were added to water bowls in which daphnids were kept. The media was refreshed every day. In terms of feeding, 1 ml of the solution obtained by dissolving 1 gram of feed in 1 liter of water was taken by feeding daphnids twice a day in the morning and evening. During the experiment, heart rates, number of eggs, survival rates/mortalities, molt cycle and number of offspring were recorded.

Method

Heart rate

All Daphnids were removed from the containers along with the water in which they were kept at the time of measurement. Each daphnia was removed with a pipette and placed on a microscope slide and viewed under a light microscope. Daphnids were recorded with a video camera and beats per minute (bpm) were counted in 7-day-interval slowed footage using a VLC player.

Number of eggs, molts and offspring

The number of eggs was recorded at 7-day intervals by counting the eggs in the dorsal brood sac. Offspring production and the number of molts were measured once every week; once counted, the offspring were removed.

Survival rates/Mortalities

The survival rates were recorded and neonates were removed from the containers at 7-day intervals until the end of the measurement periods.

Data analyses

Statistical analyses were performed using IBM SPSS Statistics 25.0 software package. Data conforming to parametric testing standards were analyzed using one-way ANOVA followed by Tukey post hoc test where different letters indicate significant differences among groups. Data not suitable for parametric tests were compared by Kruskal-Wallis test followed by Tukey post hoc test while descriptive statistics were used to demonstrate the frequencies.

Results

Heart rate

Results by various concentrations of carbamazepine on the heart rate of *D. magna* are presented in Table 1. Optical measurements from the video records of the light microscope showed that average heartbeats were similar in the both control and treatment groups in the first measurement period (Table 1). However, the control group differed from treatment groups during the 2nd, 3rd, 4th and 5th measurement periods with lower values ($p<0.05$). In parallel with the increase in drug concentration in the groups, an increase in heart rate was observed ($p<0.05$).

Table 1. Comparison of heartbeats of groups by periods

Periods	Groups	n	$\bar{x} \pm S_{\bar{x}}$	p
1	Control	60	285,73±3,21	0,951
	1,25 mg/L	60	285,67±2,47	
	1,75 mg/ L	60	287,60±2,53	
	2,25 mg/ L	60	285,68±2,99	
2	Control	60	288,40±3,74[a]	0,001
	1,25 mg/L	56	297,00±3,02[ab]	
	1,75 mg/ L	54	301,80±3,78[b]	
	2,25 mg/ L	52	308,87±3,94[b]	
3	Control	59	298,85±1,73[a]	0,000
	1,25 mg/L	56	305,29±1,98[ab]	
	1,75 mg/ L	53	311,77±2,17[b]	
	2,25 mg/ L	48	320,33±2,27[c]	
4	Control	59	298,71±1,56[a]	0,000
	1,25 mg/L	54	316,89±3,06[b]	
	1,75 mg/ L	53	326,11±1,96[c]	
	2,25 mg/ L	42	338,76±2,83[d]	
5	Control	59	299,19±1,61[a]	0,000
	1,25 mg/L	52	334,77±2,17[b]	
	1,75 mg/ L	50	338,24±1,94[b]	
	2,25 mg/ L	36	347,33±2,66[c]	

$p < 0.05$; p: ANOVA; Different letters indicate significant difference among groups by Tukey test

The number of eggs produced by periods demonstrated that the 2,25 mg/L group produced the least amount in all periods ($p<0.05$) (Fig 1, Table 2). The difference that became evident in the fourth and fifth periods resulted in an increasing numerical result in favor of the control group.

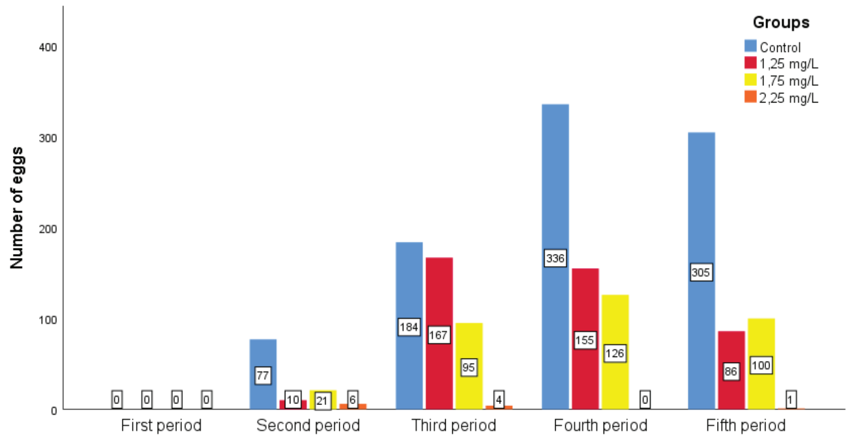

Fig 1. Number of eggs by periods

Among all groups, the fertility of the 2.25 mg/L group was adversely affected at a remarkable rate ($p<0.05$). This situation became evident during the last 3 measurement periods.

Table 2. Comparison of the number of eggs between groups by periods

Periods	Groups	n	Mean rank	Kruskal Wallis Test	Pairwise group comparisons		
					Groups	Mann Whitney U	p
1					C-5	1800.00	1.000
	Control	60	120.50	df=3 H=0.000 p= 1,000	C-7	1800.00	1.000
	1,25 mg/L	60	120.50		C-10	1800.00	1.000
	1,75 mg/ L	60	120.50		5-7	1800.00	1.000
	2,25 mg/ L	60	120.50		5-10	1800.00	1.000
					7-10	1800.00	1.000
2					C-5	1127,00	0,000
	Control	60	151,96[a]	df=3 H=44,316 p=0,000	C-7	1273,50	0,000
	1,25 mg/L	56	106,43[b]		C-10	1112,00	0,000
	1,75 mg/ L	54	117,56[b]		5-7	1627,00	0,058
	2,25 mg/ L	52	106,05[b]		5-10	1798,00	0,973
					7-10	1623,00	0,053
3					C-5	1613,50	0,824
	Control	59	126,85[a]	df=3 H=43,151 p=0.000	C-7	1224,50	0,033
	1,25 mg/L	56	130,43[a]		C-10	634,00	0,000
	1,75 mg/ L	53	103,79[b]		5-7	1131,50	0,023
	2,25 mg/ L	48	65,56[c]		5-10	507,00	0,000
					7-10	830,00	0,000

4				df=3 H=118,438 p=0,000	C-5	641,00	0,000
	Control	59	161,35[a]		C-7	379,50	0,000
	1,25 mg/L	54	106,93[b]		C-10	21,00	0,000
	1,75 mg/ L	53	95,80[b]		5-7	1251,00	0,255
	2,25 mg/ L	42	32,50[c]		5-10	231,00	0,000
					7-10	210,00	0,000
5				df=3 H=92,231 p=0,000	C-5	497,00	0,000
	Control	59	149,98[a]		C-7	495,50	0,000
	1,25 mg/L	52	84,81[b]		C-10	129,50	0,000
	1,75 mg/ L	50	96,65[b]		5-7	1092,50	0,153
	2,25 mg/ L	36	40,85[c]		5-10	429,50	0,000
					7-10	245,50	0,000

$p < 0.05$; p: Kruskal-Wallis Test; Different letters indicate significant difference among treatments by Mann Whitney U Test

Parallel to the increase in drug concentration, an increase was observed in death figures (Figure 2). The 2.25 mg/L group was the group with more deaths than the other groups.

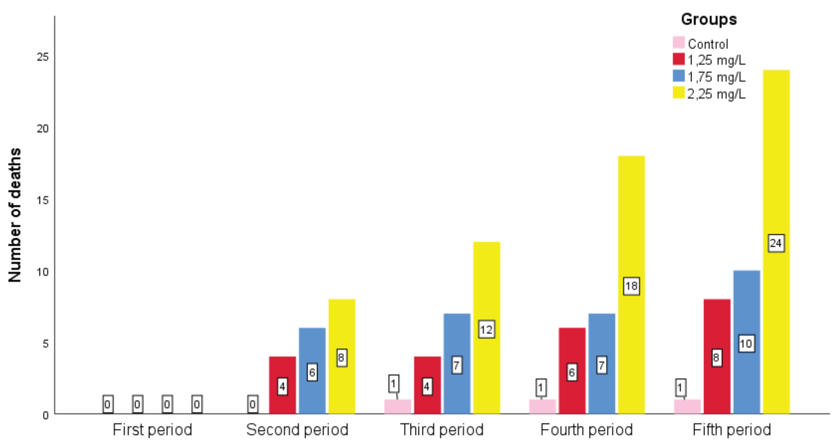

Fig 2. Cumulative mortaliy figures by periods

Molting was also affected by the amount of drug concentration. In all periods except the first period without molt, the molt number was observed more than the others in the control group. In the 2,25 mg/L group, the number of molts, which was in decline, decreased to zero in the last measurement period.

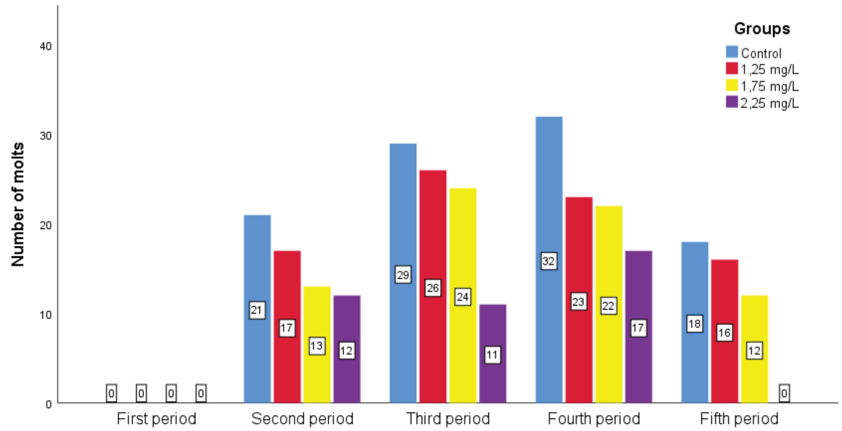

Fig 3. Number of molts by periods

The number of offspring, which was observed with low numbers in the first measurements, became evident in the last two measurement periods and a course in the opposite direction was observed with increasing drug dosages. In the 2,25 mg/L group, no offspring was found at any measurement period.

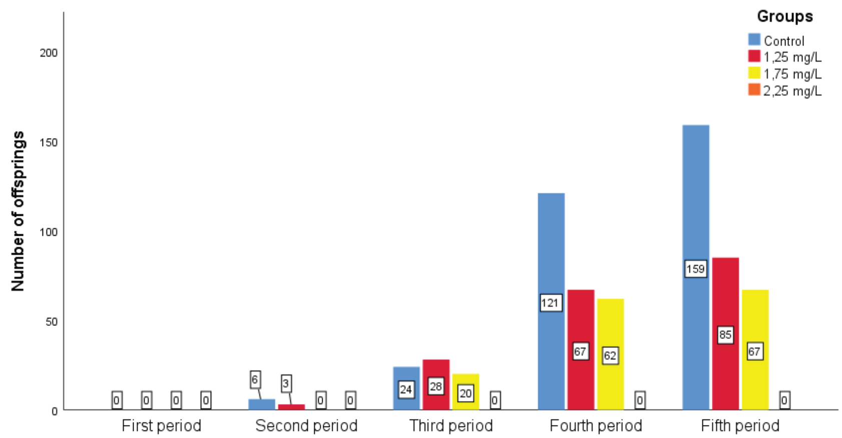

Fig 4. Number of offsprings by periods

Discussion

The present study showed that Daphnia was affected by Carpamezapine administration in terms of heart rate per minute, egg count and death figures. Research on various drugs reveals that human drugs adversely affect aquatic organisms (Allijn et al., 2018; Crane et al., 2006; Persoone et al., 2009).

Adding carbamazepine into the water in which the daphnids were kept caused an increase in heart rate ($p < 0.05$). This increase was most evident in the 2.25 mg/L group and the number of beats per minute, which was 285 beats per minute in the first measurement, increased to 347 beats per minute in the last measurement. In the literature, it is mentioned that the chemicals added to the waters have short and long-term effects on the heartbeats of daphnids (Villegas-Navarro et al., 2003). Additionally, Nkoom et al. (2019) stated that ingestion and filtration rates of the daphnids were negatively influenced by exposure to carbamazepine which might affect their growth and reproduction rates.

Considering the number of eggs produced according to the periods, it was determined that 2,25 mg/L dose administered group was clearly affected and there was a great difference between the other groups ($p<0.05$). Based on this finding, it can be stated that the chronic effect of carbamazepine causes a decrease in the number of eggs depending on the increasing dose. Similar to that result, da Silva Santos et al. (2018) reported that chronic exposure to carbamazepine resulted in changes in feeding behavior and decreased egg viability and reduced reproductive efficiency in crustaceans (Oropesa et al 2016).

The negative effects of carbamazepine application were also manifested in the number of deaths. As the dosage increased, the number of deaths also increased. More meaningful results were obtained when mortality rates were evaluated together with the number of eggs, molts and offspring.

In the current study, carbamazepine as a chemical agent had negative effects on molting. Molting is a natural biological process in Daphnia. During molting, the animal produces a new exoskeleton and sheds the old one (Song et al., 2017). This process may be adversely affected by external influences such as disrupting molting hormone signaling (Zou, 2005). Like Zou, Chen et al. (2019) reported that chronic exposure to carbamazepine can cause inhibition of molting, delayed reproduction and reduced fecundity in *D. similis*. They reported that the mean number of offspring per brood, broods per female, molts, size of the first brood and total offspring per female decreased significantly with increasing carbamazepine concentrations. These results are in accordance with the findings of this study.

On the other hand, as seen in Fig 4, carbamazepine administration caused a decrease in the number of offspring which does not coincide with the findings of Rivetti et al. (2009). They reported that carbamazepine enhanced offspring produced at environmentally relevant concentrations of 1 ng/L, 0.1 g/L and 1 g/L, respectively. When both studies are compared, it is seen that there is a serious dosage difference between them. As a matter of fact, in the current study, it is observed that the number of offspring at low dose is higher than at high dose. It is worth investigating whether lowering the dosage further will cause an increase in the number of offspring or up to which dosage will trigger this increase and then have an adverse effect. One of the things worth remembering at this point is pharmaceuticals are specifically designed to produce maximum biological activity at low doses (Fabbri and Franzellitti, 2016).

Conclusion

Carbamazepine is one of pharmaceuticals that is found at the highest frequency and amounts in the aquatic environment. Therefore, it is important to know its effects on aquatic organisms. The results of this study showed that carbamazepine poses a significant risk to *Daphnia magna*, so naturally, the same risk exists for other aquatic organisms. Taking the necessary precautions based on this information will be helpful in preventing the problem.

Acknowledgments

The authors would like to thank the Fisheries and Diseases Laboratory of the Faculty of Veterinary Medicine at Tekirdağ Namık Kemal University for the facilities provided.

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CHAPTER 2

ALLEVIATION OF PHOSPHORUS DEFICIENCY IN ORGANIC AGRICULTURE BY PHOSPHORUS SOLUBILIZING BACTERIA

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1. INTRODUCTION

Sustainable agriculture consubstantiates the disciplines of water quality and supply, food security, soil health, nutrient cycling, energy efficiency, animal and plant physiology, breeding, pest control, and ecology. Thus, it creates new ecological models by renewable and eco-friendly strategies to protect biological diversity and nutrient balance in soil (Nandwani and Nwosisi, 2016). Organic agriculture (OA), which is a major part of sustainable agriculture, aims to avoid the usage of inorganic fertilizers, synthetic pesticides, and genetically modified organisms, therefore, it seeks to reduce environmental pollution and protect the health of humans, animals and plants. Conventional agriculture threats to soil health and quality due to water pollution from fertilizers, pesticides, overuse, and wetland draining, air pollution from greenhouse gases, land degradation, salinity, and reduction of biological diversity (Tal, 2018; Kılıç et al., 2020). So, the OA has been supported by many researchers and governments and demanded by consumers in the last century (Sharma, 2001; Falguera et al., 2012; Khadda, 2021).

The emergence of the idea of the OA is based on the understanding of the damage to the environment caused by the intensive chemical inputs used in conventional agriculture. However, different sustainable and renewable practices are needed to meet successful production and minimize the risks due to the scarcity of chemical and synthetic inputs. Commonly used practices in the OA are crop rotations, using of beneficial symbiotic and non-symbiotic microorganisms to provide nutrient supplies, green manure application, utilization of organic manures and biological pest management (Tunalı et al., 2016; Monaci et al., 2017; Muneret et al., 2018; Mamiev et al., 2019; Mishra et al., 2019; El-Goud et al., 2021). Although all practices (alone or integrated) have various promotive and effective roles on different requirements of the OA, usage of plant growth-promoting rhizobacterias (PGPRs) offers a variety of benefits in many ways due to different mechanisms including nitrogen fixation, phosphorus solubilizing and mineralization, potassium solubilizing, siderophore and indole acetic acid production, 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase activity, synthesis of organic acids, hormones and vitamins. Thanks to these mechanisms, PGPRs enhance root system architecture, increase water and nutrient uptake, improve plant growth, increase systemic resistance to stress factors, therefore, they rise crop yield and quality (Ramazan, 2005; Erman et al., 2010; Hayat et al., 2012; Etesami et al., 2021; Glick and Nascimento, 2021; Santoyo et al., 2021).

Fertilization in organic agriculture is a complex and systematic process that must be managed in itself. Due to the prohibition of the use of inorganic fertilizers in organic agriculture and the limitation of the use of fertilizers even from organic sources, the effective use and protection of the existing nutrient resources per unit area are of vital importance. For example, in order to prevent agricultural nitrogen from causing water pollution, the total amount of animal manure to be used in organic plant production cannot exceed 170 kg N ha^{-1} $year^{-1}$ (Mevzuat Bilgi Sistemi, 2010). This limit is only applied in the use of farm manure, dried farm manure, dried poultry manure, composted animal manure, poultry manure, farm manure and liquid animal manure. Due to these restrictions, particularly, phosphorus (P) management has a critical role in the OA systems. P, which is a basic plant nutrient, is required for energy generation and transfer, carbon metabolism, membrane formation, enzyme activation, nitrogen fixation, formation of nucleic acids, ATP and phospholipids (Föhse et al., 1988; Marschner, 1995; Taliman et al., 2019). On the other hand, the main problem is not P deficiency in the soils but can not be uptaken by plants due to absorption by cations (Patle et al., 2019).

There are some practices based on organic amendments, however, they are generally high-cost applications and do not provide a sustainable solution. Also, it is well known that organic amendments also lead to P surplus in high usings. Thus, phosphate solubilizing PGPR strains can convert phosphorus compounds that cannot be taken up by plants into a soluble form. Thus, soil pollution can be reduced by decreasing the accumulation of P in agricultural soils, and a part of the phosphorus needed by plants can be provided by making optimum use of surplus P. This chapter aims to evaluate the possibilities of using PGPR in order to eliminate P deficiency in organic agriculture, to discuss the effectiveness of research on the subject and to examine how to create a sustainable approach.

2. PHOSPHORUS AVAILABILITY, LOSSES AND SURPLUS IN AGRICULTURAL LANDS

Phosphorus, which is the second most important macronutrient after nitrogen, restricts the growth and development of plants and reduces crop yield and quality in agricultural production (Nkaa et al., 2014; Özyazıcı and Açıkbaş, 2019; Shafi et al., 2020). For this reason, plants can benefit from a little of the applied P, since phosphorus reacts with Ca ions in alkaline soils and Fe, Al and Mn ions in acidic soils. Thus, P is fixed by cations or clay minerals or turned into organic compounds that cannot be taken up by plants (Sattari et al., 2012). Studies indicate that the approximately 5.7 billion hectares of agricultural areas show phosphorus deficiency worldwide and are increasing by day (Granada et al., 2018). Similarly, Hou et al. (2020) published a meta-analysis on the P-status of croplands and indicated that 49% of arable lands suffer from P scarcity. Except for fixation of the P, erosion, which includes various types such as soil, wind, tillage resulting from soil particles adhering to lifted crops erosion, is another phenomenon in depletion of natural resources like the P (Verheijen et al., 2009). Although quantification of

P losses due to erosion from soils is not exactly possible, Liu et al. (2008) suggested that approximately 3, 8 and 13 kg P ha^{-1} are annually lost from ordinary pastures, overgrazed pastures and arable soils, respectively.

There is another contradiction that although synthetic fertilizer in agricultural production causes depletion of 19 million tons of rock phosphate per year, only one-fifth of the can be used and converted into phytonutrient (Cordell et al., 2009). The rest of the applied P sources accumulate in the soil, thereby, it not only leads to economic losses and also causes soil and environmental pollution. According to Organisation for Economic Co-operation and Development (OECD), P surplus per unit area of agricultural lands varied between 0-20 kg ha^{-} among European countries (OECD, 2010), and average P surpluses were estimated as 8 kg ha- by Richards and Dawson (2008). Thus, research and techniques for more effective use of the existing surplus P resources in the soil play a vital role both in terms of conventional and organic agriculture without the use of inorganic fertilizers.

Organic phosphate is the main phosphorus source in the soil that comes from organic matter (OM) of animal and plant residues (Sanguin et al., 2016). Range of 4-90% of the total P in the soil comes from soil OM and plays a critical role in the recycling of P. Different researchers claim that agricultural production can be maintained in many areas in European countries without P fertilizer due to high surplus of P in soils (Macdonald et al., 2011; Schröder et al., 2011). Römer (2009) determined that most of the agricultural lands in European countries have sufficient or high P-status. However, some practices and techniques are needed for more effectively using sources.

3. PHOSPHORUS MANAGEMENT IN ORGANIC AGRICULTURE

Organic agriculture systems restrict fertilizer practices such as inorganic and synthetic inputs. European organic agriculture regulations let only two types of P-based products including rock phosphates and organic materials. The OA focuses on slurry, animal manure, composts, green manure, biological nitrogen fixation, or organic fertilizers including blood, guano, horn, bone and fishbone meals (Ceritoglu et al., 2018,2019; Reimer et al., 2020). However, acceptable P inputs in the OA are generally poorly effective (rock phosphate, bone and fishbone meals, etc.) or required high-cost for production or transportation (manure, compost and slurries). For these reasons, P management in the OA is a critical, complicated and major step.

Rock phosphates might be contaminated with cadmium and EU legislation had accepted a maximum of 90 mg cadmium per kg of P_2O_5 in rock phosphates (Nesme et al., 2014). Moreover, rock phosphate has very low solubility in soils with a pH lower than 6.0, therefore, it is not a sufficient

P source for optimal plant growth (Fardeau et al., 1998). Animal manure, which is a valuable organic amendment, is allowed to use in organic agriculture if they are obtained from farms but not from factory farming. However, due to the questioning of the reliability of the sources from which the animal manure is supplied, it has been desired to remove their use in the OA in many developing countries, especially in Europe. Although Denmark tried to take the first steps on eliminating the use of animal manure and straw in the OA, it had to postpone its decision up to 2022 due to doubts about sustainable P management (Oelofse et al., 2013). Thus, the use of phosphate solubilizing bacteria (PSB), which increases the mineralization of organic phosphorus resources in the soil and promotes the uptake and transport of available phosphorus by plant roots, is an increasing phenomenon in both conventional and organic agriculture.

4. ACTION MECHANISM AND APPLICATION PRACTICES OF PHOSPHORUS SOLUBILIZING BACTERIA

PGPR can be described as bacterial strains increasing water and nutrient uptake, gaining nitrogen and phosphorus to plants by biological nitrogen fixation and phosphate mineralization and promoting plant growth. Besides, PGPR increases stress tolerance due to mechanisms such as secretion of various phytohormones, vitamins and growth regulators, restriction of ethylene synthesis with ACC deaminase activity, decreasing of pathogen damage by the secret of antibiotic and fungicidal compounds (Glick, 2020; Chowdhury, 2022). Ideal PGPR strain should involve some criteria including eco-friendly, high colonization in the rhizosphere, promotion of plant growth, an exhibition with a wide spectrum of action, high competition with other bacterias, tolerant to biotic and abiotic stress factors (Basu et al., 2021). PSB strains that can solubilize insoluble P_i compounds such as hydroxyapatite, dicalcium phosphate, tricalcium phosphate, and rock phosphate, and mineralize organic phosphate sources to useful forms by plants (i.e. $H_2PO_4^-$ and HPO_4^{-2}), belong to different genera including *Bacillus*, *Agrobacterium*, *Chryseobacterium*, *Pseudomonas*, *Xanthobacter*, *Achromobacter*, *Actinomadura*, *Aerobactor*, *Alcaligenes*, *Arthrobacter*, *Enterobacter*, *Delftia*, *Klebsiella*, *Gordonia*, *Phyllobacterium*, *Pantoea*, *Rhodococcus*, *Thiobacillus*, *Streptomyces*, *Serratia*, *Xanthomonas* (Rodriguez and Fraga, 1999; Chen et al., 2006; Zaidi et al., 2009; Wang et al., 2021).

There are three main processes for the P cycle in soils including mineralization-immobilization, dissolution-precipitation and sorption-desorption. Mineralization of organic sources and solubilization of all phosphorus sources have a pivotal role on phosphorus uptake by plants (Alori et al., 2017; Etesami, 2020; Saha et al., 2021). PSB uses different mechanisms to increases phosphorus uptake by plants or copes with P deficiency such as production of organic and inorganic acids, siderophore production, indole-3 acetic acid (IAA) production and ACC deaminase activity.

Although P uptake is commonly realized under neutral pH (6.5-7.0) due to balance reactions, PSB can solubilize inorganic phosphate compounds in neutral to alkaline soils by secreting protons (H^+) and organic/inorganic acids, therefore, it maintains electroneutrality in the rhizosphere (Jones and Oburger, 2011). The basic organic acids secreted by PSB are aspartic, 2-ketogluconic, citric, gluconic, malic, lactic, succinic, malonic, oxalic, and tartaric acid (Mardad et al., 2007; Chen et al., 2016; Yin et al., 2020). The main action of organic acids on P solubilizing depends on ligand exchange (Jones and Oburger, 2011). PSB both improves soil P level by lowering rhizosphere pH and also compete with phosphate compounds to bind clay surface (Bolan et al., 1994). In addition, PSB provides various signals that fetch different microbial organisms in the rhizosphere, thereby, contributing plant growth, nutrient uptake and increasing systemic resistance to adverse environmental conditions (Backer et al., 2018).

It is known that genotypic properties have a pivotal role on root system architecture, therefore, it influences water and nutrient uptake and so plants development, quality and yield (Nguyen and Stangoulis, 2019; Shao et al., 2019; Ceritoglu et al., 2020; Liu et al., 2021). Out of genotypic properties, environmental factors and various chemicals such as phytohormones or root exudates leads to change root architecture. Many PGPR strains can secret auxin (Gupta et al., 2015), IAA (Afzal et al., 2015), cytokinins and gibberellins (Kumar et al., 2015). While auxins secreted by plants and PGPR provide longer root structure, cytokinins involve interaction with auxins and cause transcriptional fluctuations on hormone synthesis, defense systems and cell wall formations, therefore, nutrient uptake is affected to a major degree (Spaepen et al., 2014).

Solanki et al. (2020) stated that the application of PSB promoted the number of branches (11.81%), plant height (14.92%), nodule dry weight (11.95%), number of nodules (8.19%), number of pegs (18.75%), number of total pods (6.61%), number of mature pods (14.99%), test weight (8.38%), shelling percentage (6.31%), pod yield (14.99%), protein content (7.62%) and haulm yield (14.78%) in groundnut compared with control plants. Tahir et al. (2018) indicated that the PSB enhanced the performance of bread wheat under low fertilizer and arid climate conditions, but, it was more effective if using combined with bio-organic phosphate. They stated that co-application of bio-organic phosphate and PSB induced the grain yield of cv.Galaxy-2013 and cv.Punjab-2011 by 54.3% and 83.3, respectively. EL Maaloum et al. (2020) determined that consortia that consist of phospho-compost, PSB and AMF increased dry matter accumulation in shoot and total root biomass up to 239% and 395%, respectively. Liu et al. (2021) conducted a volatile experiment including broth culture, soil incubation and pot processes in which PSB (*Bacillus thuringiensis* and *Pantoea ananatis*) can solubilize $Ca_3(PO_4)_2$, $FePO_4$, and $AlPO_4$ as a P source and promoted water-soluble phosphate concentrations. Aye et al. (2021) PSB inoculation with phosphorus fertilizers improved the yield from 17.03 to 38.42% and increased the percentage of sugar cane from 4.8 to 9.96% over control plants. Wahid et al. (2020) laid out an experiment on the impacts of PSB and AMF on phosphorus uptake under calcareous soil conditions and determined that co-inoculation of AMF and PSB significantly improved P uptake and yield of maize-wheat plants. Karaman et al. (2009) detected the inoculation of chickpea seeds with *Rhizobium* spp. promoted residual P use efficiency of following wheat plants. Suleman et al. (2018) indicated that gluconic acid-producing PSB (*Pseudomonas* sp.) enhanced phosphorus uptake of wheat via activating glucose dehydrogenase gene expression. Adnan et al. (2020) denoted that PSB inoculation increased P acquisition and use efficiency in maize by secreting organic and inorganic acids and increasing phosphatase activity under lime-induced salinity conditions.

5. CONCLUSION

Phosphorus has a critical position in organic agriculture due to its immobile structure, absorption by cations, low acquisition by plants and limited organic phosphorus sources. Thus, utilization by available organic and inorganic phosphorus sources in soil plays a pivotal role for plants cultivated in organic agriculture. Phosphorus solubilizing bacteria is one of the most important strategies for mineralization of phosphate sources and converting them into available form in the rhizosphere. PSB using also starts up as low-cost and eco-friendly properties.

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CHAPTER 3

GRAFTING ENHANCES MELON DROUGHT TOLERANCE BY IMPROVING ION REGULATION AND ANTIOXIDANT DEFENSE SYSTEMS

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In addition to the increasing impact of climate change on natural water resources, the shifting global pattern of precipitation (Dai, 2013) and the growth of agricultural operations, which affect the dynamics of soil moisture, have increased the frequency and severity of droughts throughout the world. Approximately one-fourth of the Earth's surface is covered by arid and semiarid land masses, but droughts can occasionally occur in other regions. (Zhang et al., 2017; Sati et al., 2022). As a result, drought disruption is expected to reduce crop production by 36%, though yield reductions in various crops can range from 13% to 94%, depending on the duration and intensity of the stress (Heikal et al., 2022).

Drought stress is a form of environmental stress that has a substantial influence on biochemical, molecular, and physiological changes in plants, all of which are detrimental to their growth and development (Qie et al., 2019). Plants evolve a variety of strategies in response to drought stress, including the up- or down-regulation of certain genes, a transient rise in abscisic acid, the buildup of suitable solutes and protective enzymes, enhanced antioxidant levels, and controlled energy-consuming processes.

Abiotic stressors, such as drought, generate an excess of reactive oxygen species (ROS), which can result in protein oxidation, lipid peroxidation, enzyme deactivation, DNA damage, and/or interaction with other essential plant cell components. Plants use a variety of enzymatic and nonenzymatic antioxidative processes to counteract the negative consequences of reactive oxygen species (ROS). Enzymatic antioxidants include superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR), while nonenzymatic antioxidants consist of reduced glutathione (GSH), ascorbate, carotenoids, and tocopherol (Kusvuran et al., 2016).

Over recent decades, there have been several strategies proposed and used to minimize the effects of abiotic stress on productivity in the field of agriculture and one possible solution to cope involves using graft technology. Breeding of resistant genotypes has also been a method used to aid crops such as horticultural crops in improving their resistance to drought stress; however, it is a laborious and complex process because of the polygenic nature of a plant's resistance to drought. As a result, developing novel methods for producing plants that are more resistant to the impacts of drought stress remains a top concern (Ezzo et al., 2020).

It has been suggested that rootstock grafting may modify the hormone balance in scions, since it alters the scion's features, such as plant hydraulics and water and nutrient intake, which are altered by vascular connection disturbances (Fullana-Pericas et al., 2018). As a result, grafting is a frequently used technique for increasing plants' salt tolerance by grafting a productive scion onto a tolerant rootstock, or for physically initiating tolerance to stress (Simpson et al., 2015). Grafting is regarded as a critical technique for mitigating the effects of abiotic stress factors, notably in tomato and melon (Penella et al., 2016). Melon (*Cucumis melo* L.), a member of the Cucurbitaceae family, is a prominent horticultural crop farmed mostly in arid and semiarid locations where salt stress remains a significant issue, despite the improved management strategies established over the last decades (Ulas et al., 2019).

The current study studied the effects of grafting the physiological and metabolic reactions of plants to drought using salt/drought-tolerant melon genotypes 'TLR-1 and TLR-2' and 'Albatros' as rootstocks and salt/drought-sensitive melon genotypes 'SCP-1 and SCP-2' as scions. The study assessed the drought sensitivity of melon seedlings that had been grafted with a drought-tolerant rootstock and laid the groundwork for grafting-based drought tolerance improvement. Additionally, the relationship between grafting and improved drought resistance was discovered. Thus, an examination of the efficacy of grafting in increasing drought tolerance in melon, a significant species in arid and semiarid environments, was done herein.

Material & Methods

Plant materials and growth conditions: Three rootstocks were used: TLR-1 and TLR-2, which were selected as salt/drought-tolerant melon genotypes in a previous study by Kusvuran (2012), and Albatros (*Cucumis melo*), a commercial rootstock from Rito Seeds Company in Turkey. SCP-1 and SCP-2, both of which are salt/drought-sensitive melon genotypes, were employed as the scion. All rootstocks were grafted with SCP-1 and SCP-2 scions, yielding the following graft combinations: SCP-1/TLR-1, SCP-1/TLR-2, SCP-2/TLR-1, SCP-2/TLR-2, and SCP-1/Albatros. The control treatment included two genotypes of *C. melo* (SCP-1 and SCP-2), which were not grafted and were allowed to establish their own roots. Grafting was carried out using LEE's 1994 single cotyledon approach. Both grafted and ungrafted plants were repotted into 12-L plastic pots with a 2:1 combination of peat and perlite. The plants were then maintained under greenhouse conditions which comprised day/night temperatures of 26 ± 2 °C and 18 ± 2 °C, with a relative humidity of 65% ± 5. Each pot included four plants, and each experiment consisted of four replications. All treatments received consistent optimum irrigation (100% FC) for 18 days after transplanting to promote root system development without water stress; during the first 18 days after planting a nutrient solution, 30% drainage was ensured to prevent saline accumulation in the substrate. In this experiment, drought stress treatments were administered 18 days after transplanting and continued for 12 days. 100% field capacity (FC) irrigation was applied to the control treatment, whereas mild water stress equated to 25% field capacity (25% of the available water-holding capacity). Upon conclusion of the experiment, evaluation of the plants was performed via morphological, physiological, and biochemical parameters.

Relative water content (RWC) and leaf water potential (Ψw): The RWC was estimated using the method of Türkan et al. (2005). A pressure chamber was used to measure the leaf water potential of the third completely expanded leaf over the center of the photoperiod (Muries et al., 2013).

Ion concentration: To determine the ion concentration, the melon leaves and roots were dried at 65 °C for 48 hours. Following that, the samples were ground in a mill fitted with a 20-mesh screen. For approximately 6 hours at 550 °C, the leaf powder was converted to ash, which was subsequently dissolved in 3.3 percent HCl. The amounts of Na, K, Ca, Fe, Mn, and Zn in the roots and leaves were determined by atomic absorption spectrometry. The Cl content in tissue samples was evaluated using the Mohr technique using titrimetric analysis with silver nitrate ($AgNO_3$) (Dasgan et al., 2018).

Malondialdehyde (MDA) content: The lipid peroxidation rate was calculated using the MDA concentration obtained from the thiobarbituric acid (TBA) reaction of the supernatant at 10,000 g and 4 °C for 10 minutes. Absorbance at 532 nm was measured, and data that corresponded to non-specific absorption at 600 nm were eliminated (Heath and Packer, 1968).

Photosynthetic pigments*:* The total carotenoid, chlorophyll a (Chla), and chlorophyll b (Chlb) concentrations were determined using Arnon's (1949) method. The absorbance of the pigment extraction was measured at 663, 645, and 470 nm using a Shimadzu UV mini-1240 spectrophotometer (Kyoto, Japan). The pigment was extracted using 80% (v/v) acetone.

Antioxidant enzyme activities: Using a mortar and pestle and 5 mL of extraction solution containing 50 mM potassium-phosphate buffer, pH 7.6, and 0.1 mM disodium ethylenediaminetetraacetate, enzymes were extracted from 0.5 g of leaf tissue. The homogenate was centrifuged at 15,000 *g* for 15 minutes, and the supernatant fraction was used for the enzyme assay. All preparatory steps for the enzyme extraction were carried out at a temperature of 4 °C. Cakmak and Marschner (1992) established the SOD test by detecting the reduction of superoxide radical (O^{2-})-induced nitro blue tetrazolium at 560 nm. The CAT activity was determined by monitoring the disappearance of hydrogen peroxide. To evaluate the APX activity, the ascorbate intake was determined using its absorbance at 290 nm. 1 unit of APX activity was defined as the quantity of enzyme necessary to consume 1 mol of ascorbate min^{-1} (Cakmak and Marschner, 1992). By measuring the absorbance of nicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm and its enzyme-dependent oxidation, the GR activity was measured. 1 unit of GR activity was defined as the volume of the enzyme that oxidized 1 mol of NADPH min^{-1}.

Statistical analysis: The experiment employed a totally randomized plot design with four replicates. The Tukey multiple range test was used to compare the mean values. $P<0.05$ was determined statistically significant using SPSS version 13.0 for Windows (SPSS Inc., Chicago, IL, USA). In all figures, data are provided as mean standard deviation (SD), and error bars reflect standard errors of the means.

Results

Drought stress (25% FC) significantly decreased all morphological parameters (i.e., shoot and root fresh and dry weight, leaf area) of grafted and ungrafted melon plants (Table 1). Drought severely decreased the development of ungrafted melon seedlings, but drought stress had a less severe effect on grafted seedlings. Under drought stress, the fresh and dry weights of the shoot and root, as well as the leaf area, of ungrafted seedlings were reduced by 60%, 66%, 60%, 44%, and 78%, respectively, whereas they were reduced by 29%, 27%, 26%, 23%, and 28%, respectively, in grafted plants. Additionally, all of the plants treated with water stress, SCP-1/TLR-2, SCP-2/TLR-2, SCP-1/Albatros, and SCP-2/Albatros, demonstrated the least loss in growth parameters of all grafted combinations.

The combination between grafting and drought treatment resulted in a significant change in RWC and leaf Ψw at the $P < 0.001$ level. As melon seedlings were subjected to drought stress, their RWC reduced by 49% and by 51% in ungrafted plants, respectively, when compared to controls (Table 1). However, when drought-stressed ungrafted plants were compared to grafted plants, considerably enhanced RWC was seen at rates of 63% and 71%. When water stress was applied, the greatest improvement in RWC was reported with the Albatros rootstock combination for both SCP-1 and SCP-2, where the increase was 71% and 81%, respectively. The leaf w was considerably altered by drought stress, ranging from –0.36 to –0.68 MPa. These findings indicated that grafting had a beneficial effect on the Ψw in conditions of stress (increase of 38% and 31%).

Table 1. *Effects of rootstock on the growth parameters of the melon seedlings under drought stress*

Graft Combination	T	Shoot fresh weight (g plant^{-1})	Shoot dry weight (g plant^{-1})	Root fresh weight (g plant^{-1})	Root dry weight (g plant^{-1})	Leaf area (cm^2 plant^{-1})	RWC (%)	Leaf Ψw
SCP-1	C	$188.74\pm8.27^{e-h}$	$20.52\pm3.32^{e-g}$	5.69 ± 0.43^{ef}	$0.36\pm0.04^{f-h}$	3272.18 ± 20.44^{fg}	85.62 ± 8.78^{a}	-0.19 ± 0.01^{a}
	D	54.24 ± 4.51^{i}	7.48 ± 0.96^{h}	2.22 ± 0.13^{i}	0.21 ± 0.01^{h}	844.80 ± 38.82^{i}	43.15 ± 2.81^{d}	-0.68 ± 0.05^{f}
SCP-1/TLR-1	C	$223.00\pm14.30^{c-g}$	$23.42\pm1.34^{d-f}$	$6.12\pm0.51^{d-f}$	$0.42\pm0.02^{e-h}$	3515.63 ± 70.04^{ef}	81.94 ± 2.27^{a}	-0.20 ± 0.03^{a}
	D	141.24 ± 6.27^{h}	14.87 ± 1.11^{g}	4.17 ± 0.07^{h}	0.32 ± 0.03^{gh}	2317.83 ± 45.86^{h}	62.55 ± 2.73^{bc}	-0.51 ± 0.03^{de}
SCP-1/TLR-2	C	$236.59\pm10.44^{c-f}$	27.98 ± 1.22^{cd}	7.67 ± 0.39^{ab}	$0.64\pm0.10^{d-f}$	4859.62 ± 78.22^{ab}	83.69 ± 5.78^{a}	-0.23 ± 0.01^{a}
	D	$170.54\pm6.74^{f-h}$	$23.16\pm0.49^{d-f}$	5.24 ± 0.29^{fg}	$0.52\pm0.03^{d-g}$	3958.51 ± 41.38^{de}	$74.00\pm1.95^{a-c}$	-0.39 ± 0.07^{bc}
SCP-1/ Albatros	C	305.12 ± 13.88^{ab}	30.35 ± 1.81^{bc}	7.73 ± 0.32^{ab}	0.67 ± 0.04^{de}	$4601.82\pm99.52^{a-c}$	82.33 ± 4.76^{a}	-0.18 ± 0.03^{a}
	D	$229.04\pm11.79^{c-f}$	$25.94\pm1.14^{c-e}$	5.66 ± 0.38^{ef}	$0.56\pm0.03^{d-g}$	3645.29 ± 54.65^{ef}	$73.96\pm2.64^{a-c}$	-0.36 ± 0.01^{b}
SCP-2	C	$215.65\pm6.81^{c-g}$	$24.14\pm1.49^{c-f}$	$6.31\pm0.48^{c-e}$	0.75 ± 0.11^{d}	$4412.16\pm84.52^{b-d}$	84.96 ± 3.59^{a}	-0.24 ± 0.03^{a}
	D	37.02 ± 3.73^{i}	6.86 ± 0.15^{h}	2.60 ± 0.26^{i}	$0.40\pm0.04^{e-h}$	806.65 ± 44.38^{i}	41.11 ± 3.78^{d}	-0.70 ± 0.02^{f}
SCP-2/TLR-1	C	$258.77\pm13.28^{a-d}$	28.15 ± 3.23^{cd}	$6.98\pm0.46^{b-d}$	1.09 ± 0.15^{c}	4715.79 ± 107.65^{ab}	82.06 ± 3.48^{a}	-0.21 ± 0.01^{a}
	D	159.55 ± 8.72^{gh}	18.32 ± 0.55^{fg}	4.51 ± 0.24^{gh}	0.74 ± 0.03^{d}	2850.43 ± 41.26^{gh}	61.93 ± 1.37^{c}	-0.54 ± 0.04^{e}
SCP-2/TLR-2	C	$277.71\pm18.61^{a-c}$	36.11 ± 0.62^{ab}	8.09 ± 0.35^{a}	1.49 ± 0.35^{ab}	5160.56 ± 104.59^{a}	84.28 ± 4.77^{a}	-0.20 ± 0.03^{a}
	D	$204.07\pm13.05^{d-h}$	$26.35\pm1.94^{c-e}$	6.61 ± 0.22^{cd}	1.11 ± 0.05^{c}	$3968.96\pm53.79^{c-e}$	$73.78\pm2.79^{a-c}$	-0.45 ± 0.01^{cd}
SCP-2/ Albatros	C	308.18 ± 10.46^{a}	39.88 ± 2.92^{a}	8.36 ± 0.53^{a}	1.75 ± 0.11^{a}	4856.42 ± 133.16^{ab}	81.84 ± 3.64^{a}	-0.20 ± 0.02^{a}
	D	$240.57\pm5.25^{b-e}$	28.11 ± 0.95^{cd}	7.04 ± 0.20^{bc}	1.35 ± 0.05^{bc}	3227.52 ± 65.06^{fg}	74.53 ± 3.29^{ab}	-0.40 ± 0.02^{bc}

*Each value represents the mean of four replicates. For each parameter, different superscripted letters represent statistically significant differences at $P< 0.05$ according to the Tukey test (T: treatment, D: drought, C: control).

As indicated in Figure 1 and Table 2, grafting was assessed for its potential to enhance the accumulation of K, Ca, Mg, Zn, Mn, and Fe in melon plants under drought stress. The accumulation of macro and micro nutrients decreased significantly in drought-stressed plants. However, total findings demonstrated that grafting contributed significantly to the mineral uptake of water-deficient melon plants. Compared to the C group of ungrafted plants, these decreases were determined to be between 26% and 65% under drought stress. Grafting had a favorable effect on the nutrient content, and substantially higher leaf and root macro and micro ion levels (9–214%) were recorded in all grafted plants compared to the ungrafted group.

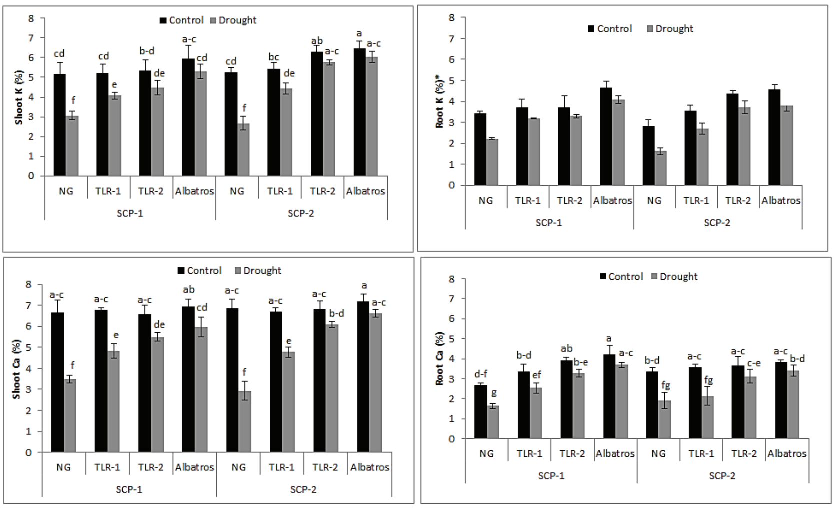

Figure 1. *Effects of non- grafting (NG) and grafting on the K and Ca contents in melon under drought stress. Each value represents the mean of four replicates. For each parameter of each different letters are significantly different at P< 0.05 according to Tukey test.*

Table 2. *Effects of rootstock on the nutrient contents of the melon seedlings under drought stress*

Graft Combination	T	Mg (%)		Fe (ppm)		Mn (ppm)		Zn (ppm)	
		Shoot	Root	Shoot	Root	**Shoot^{ns}**	**Root**	**Shoot**	**Root**
SCP-1	C	0.63±0.09c-f	0.33 ± 0.02^{cd}	67.74±7.51	$91.29\pm9.63^{b-e}$	43.73±1.68	44.89 ± 0.56^{g}	$39.09\pm0.79^{e-g}$	40.96 ± 1.78^{ef}
	D	0.33±0.04i	0.22 ± 0.02^{ef}	47.71±8,90	38.94 ± 2.65^{g}	27.45±0.94	29.80 ± 0.60^{h}	18.12 ± 1.53^{h}	30.47 ± 0.81^{h}
SCP-1/ TLR-1	C	0.58±0.08e-g	$0.36\pm0.03^{b-d}$	95.02±8,11	94.85 ± 4.49^{bc}	50.62±3.36	$60.86\pm3.18^{a-e}$	$46.36\pm1.27^{c-e}$	$46.18\pm1.23^{c-e}$
	D	0.45±0.03g-i	0.28 ± 0.04^{de}	79.66±7,36	73.84 ± 4.59^{f}	40.71±3.08	$52.16\pm1.49^{d-g}$	31.36 ± 2.51^{fg}	39.03 ± 1.91^{fg}
SCP-1/ TLR-2	C	0.61±0.04d-g	$0.35\pm0.03^{b-d}$	136.35±9,79	$91.47\pm4.83^{b-e}$	46.54±2.23	68.87 ± 1.95^{a}	68.96 ± 3.45^{a}	50.24 ± 3.38^{bc}
	D	0.53±0.02f-h	0.31 ± 0.01^{cd}	123.64±6,41	76.24 ± 6.00^{ef}	39.47±1.91	$61.95\pm1.14^{a-c}$	$56.93\pm3.73^{a-c}$	$47.20\pm1.54^{c-e}$
SCP-1/ Albatros	C	0.78±0.09a-c	0.48 ± 0.03^{a}	87.21±9,09	$91.08\pm3.27^{b-f}$	60.59±2.17	63.92 ± 2.28^{ab}	$55.56\pm2.02^{b-d}$	52.26 ± 3.80^{bc}
	D	0.70±0.03b-e	0.42 ± 0.04^{ab}	76.85±8,20	$81.15\pm4.57^{c-f}$	56.41±3.34	$56.28\pm0.93^{b-f}$	$47.61\pm2.01^{c-e}$	$46.80\pm2.11^{c-e}$
SCP-2	C	0.81±0.05ab	0.37 ± 0.07^{bc}	78.2±6,18	98.27 ± 8.21^{bc}	36.17±0.84	$51.62\pm2.66^{e-g}$	$53.36\pm3.46^{b-d}$	$47.94\pm2.94^{c-e}$
	D	0.38±0.05hi	0.18 ± 0.01^{f}	47.27±5,49	51.14 ± 1.36^{g}	24.99±2.71	28.51 ± 1.13^{h}	26.73 ± 1.13^{gh}	32.23 ± 1.27^{gh}
SCP-2/ TLR-1	C	0.78±0.05a-c	0.39 ± 0.01^{bc}	92.51±6,58	$76.86\pm4.51^{d-f}$	40.84±2.38	$55.56\pm2.69^{b-f}$	55.72 ± 1.30^{bc}	52.94 ± 3.17^{bc}
	D	0.53±0.14f-h	0.28 ± 0.03^{de}	75.25±5,82	56.15 ± 7.91^{g}	30.71±0.55	45.36 ± 1.43^{g}	$43.14\pm1.20^{d-f}$	$42.65\pm1.03^{d-f}$
SCP-2/ TLR-2	C	0.80±0.04ab	0.44 ± 0.02^{ab}	106.36±6,41	$93.79\pm5.29^{b-d}$	53.30±2.21	$61.50\pm3.77^{a-d}$	63.35 ± 1.05^{ab}	56.31 ± 2.36^{b}
	D	0.67±0.07b-f	$0.40\pm0.01^{a-c}$	91.23±6,62	75.16 ± 6.88^{ef}	46.85±3.15	$54.21\pm4.35^{c-g}$	$51.07\pm3.53^{b-e}$	48.64 ± 2.88^{cd}
SCP-2/ Albatros	C	0.89±0.03a	0.43 ± 0.05^{ab}	100.72±5,29	133.31 ± 2.35^{a}	58.61±4.21	$57.36\pm3.09^{b-f}$	61.27 ± 2.89^{ab}	66.80 ± 2.94^{a}
	D	0.75±0.04a-d	$0.37\pm0.05^{b-d}$	84.89±5,91	106.62 ± 8.27^{b}	52.21±2.83	48.45 ± 1.91^{fg}	$53.98\pm4.30^{b-d}$	56.45 ± 2.99^{b}

*Each value represents the mean of four replicates. For each parameter, different superscripted letters represent statistically significant differences at $P< 0.05$ according to the Tukey test (T: treatment, D: drought, C: control) (ns: no significant).

The intercellular levels of MDA, key stress indicator, were measured to corroborate the drought-induced oxidative stress conditions. After 12 days of drought stress, the MDA concentration was found to have risen considerably in ungrafted plants ($P<0.05$). The ungrafted SCP-2 genotype had the greatest MDA concentration (25.19 mol g^{-1} FW) following drought stress. MDA concentrations were lowest under drought stress in the SCP-1/Albatros combination (13.75 mol g^{-1} FW). When the MDA content was considered, the ratio of MDA increased by 407% and 544% when compared to controls in the ungrafted sensitive genotypes, while this rate ranged between 356–384% when grafted with the tolerant genotypes, and a decrease of 35% and 34% occurred when grafted with the tolerant genotypes (Figure 2).

The photosynthetic pigments Chla, Chlb, and carotenoids of the ungrafted melon plants were decreased by 68%, 49%, and 57%, respectively, due to drought stress. On the other hand, the Chla, Chlb, and total carotenoid contents were less affected by drought stress in the SCP-1 and SCP-2 that had been grafted with rootstock (decrease of 9-37%) (Figure 2).

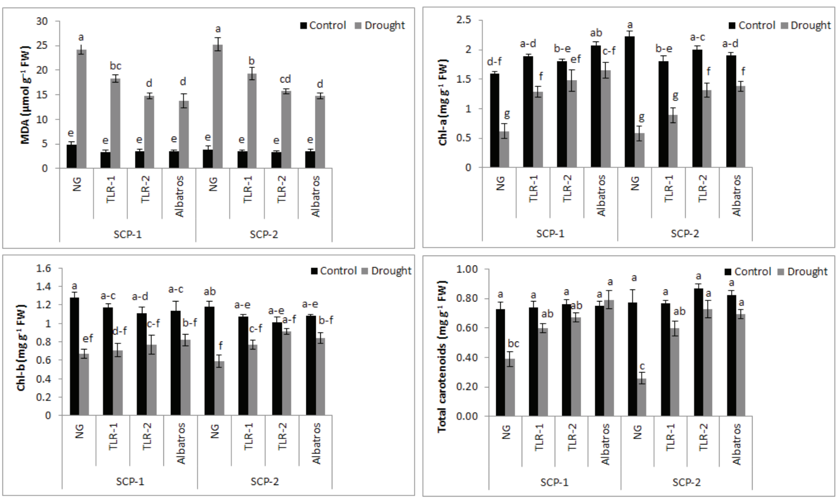

Figure 2. *Effects of non- grafting (NG) and grafting on the MDA, Chla, Chlb, and total carotenoids contents in melon under drought stress. Each value represents the mean of four replicates. For each parameter of each different letters are significantly different at P< 0.05 according to Tukey test.*

In response to water stress, the antioxidant enzyme activities (SOD, CAT, GR, and APX) increased in both the grafted and ungrafted plants. Moreover, the grafted plants exhibited much higher antioxidant enzyme activities than the ungrafted plants (Figure 3). As shown in Figure 3, the activities of antioxidative enzymes such as SOD, CAT, GR, and APX were upregulated and elevated by an average of 29, 26, 57, and 17% in the ungrafted group compared to the control group. Compared to the C and D groups, the grafting had significant and observable beneficial impacts on the sensitive genotypes. Compared to the D group, the enzyme activity (SOD, CAT, GR, and APX) in the grafted groups rose by an average of 34-138, 35-130, 44-124, and 16-55%, respectively. In addition to this, the TLR-2 and Albatros rootstock was noted as a rootstock that exhibited the highest antioksidatif enzyme activity in the SCP-1 and SCP-2 plants.

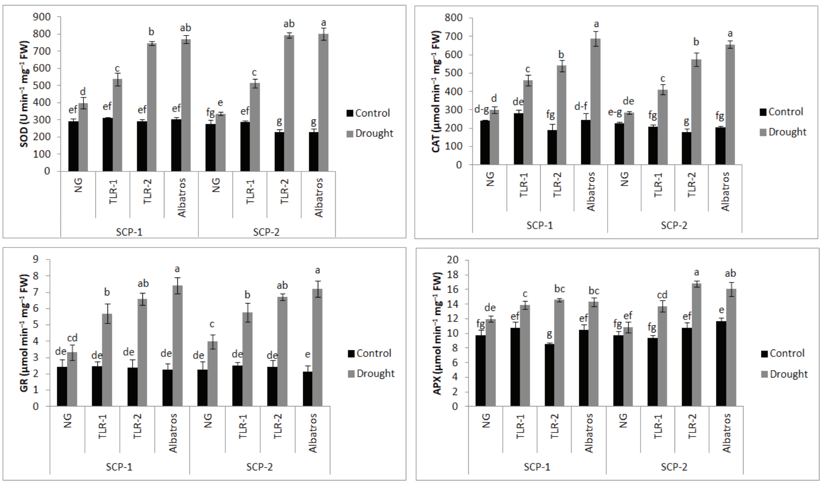

Figure 3. Effects of non- grafting (NG) and grafting on the SOD, CAT, GR, and APX enzyme activity in melon under salt stress. Each value represents the mean of four replicates. For each parameter of each different letters are significantly different at P< 0.05 according to Tukey test.

Discussion

Early growth stages are dominated by cell division, differentiation, and elongation, all of which are adversely impacted by drought stress due to a reduction in cell turgor pressure and other physiological processes (Pandey et al., 2019). In this study, grafting successfully alleviated the negative effects of drought stress on the growth and development of melon seedlings. The drought-tolerant grafted plants had shoot and root fresh and dry weights and leaf areas that were closer to those of the control plants than those of the ungrafted plants when subjected to water stress. Sanchez et al. (2012) revealed that higher tolerance in grafted plants to moderate water shortage was a drought-adaptive response primarily associated with tolerance rootstock, which results in improved plant growth and output. By delivering water and nutrients up to the stem, the roots of a plant are critical to its development, growth, and survival. It may be inferred that using salt/drought resistant rootstocks can be helpful when under the effect of salt/drought stress. Water stress has a greater effect on leaf area expansion than on photosynthesis and transpiration (Sharma et al., 2019). When plants are subjected to drought stress, their leaf area often decreases, which might be a technique employed to limit the quantity of water lost via transpiration. Changes in the cell wall and leaf turgor properties, as well as a drop in photosynthetic rates, are all examples of distinct reactions to salt/drought stress, all of which result in a decrease in the leaf's total area (Acosta-Motos et al., 2017). According to the results of this study, when compared to the control plants, it was observed that the leaf area was considerably reduced under the influence of drought stress. This effect was more influential in the ungrafted plants when compared to the grafted plants. It was reported by Böhm et al. (2017) that the highest tolerance against salt stress was seen in grafted plants with watermelon, with an improvement observed in the plant biomass and leaf area, not a reduction, due to salinity. Colla et al. (2010) observed increased dry weight values and a greater number of leaves in all of the grafted watermelon plants in their study when compared to the ungrafted control group, and it was stated by Penella et al. (2016) that a reduction in the leaf area of ungrafted plants was observed when they were grown under stress, and this condition did not change in any of the combinations of grafted pepper plants. Hence, grafting can reduce the effects of stress on melon growth by drought. The results showed that grafting the plants onto salt/drought-tolerant TLR-1 and TLR-2 was a successful strategy to improve the tolerance of the salt/drought-sensitive melon seedlings (Table 1). Additionally, grafting plants onto drought-tolerant types significantly increased the stress tolerance of melon seedlings, a trait that was also observed in tomato, eggplant, and cantaloupe (Zhu et al., 2008).

The RWC is one of the simplest agricultural parameters to utilize when screening plants for drought tolerance (Nxele et al., 2017). When transpiration exceeds water absorption, cell turgor decreases, resulting in decreased RWC and cell volume. Low RWC and turgor retard plant development, resulting in a reduction in stomatal conductance (Hammad and Ali, 2014). Plant regulation Ψw is essential not just for influencing the plant's response to abiotic stress, but also for its effect on metabolic processes, such as turgor-driven cell expansion during plant growth (Martinez-Vilalta and Garcia-Forner, 2016). Plants stressed by salt and water deficiency exhibit moderate dehydration, as shown by a decrease in Ψw, since water absorption is more difficult and the soil solution contains less accessible water (Acosta-Motos et al., 2017). The exposure of plants to water stress and/or salt stress resulted in the greatest negative values for the leaves and stems of the plants, because both passive hydration and salt gathering caused a reduction in the Ψw of the leaves. In order to modify the osmotic potential, the inorganic ion intake required less energy than the production of organic molecules within the cells (Penella et al., 2015). According to Penella and Calatayud (2018), the rootstock characteristics influenced the scions' salt tolerance because stomatal functions were successfully managed, resulting in a shift in the hormonal signaling of plants from the roots to the shoots. Under conditions of salt stress, it was shown that grafted cucumber plants had better rates of photosynthesis and stomatal conductance than ungrafted cucumber plants (Yang et al., 2006). Both stomatal conductivity and leaf Ψw values were lowered in prior research; however, the reduction was slightly less in the grafted plants under water stress. (Kıran et al., 2017).

It is widely recognized that water stress reduces the plant ionome by limiting mineral nutrient intake and translocation via lower transpiration rate, active transport, and membrane permeability. Numerous rootstocks are capable of enhancing nutrient absorption and translocation (Kumar et al., 2017). Potassium is required for proper osmoregulation and confers drought resistance. Increased K levels resulted in a decrease in stomatal conductance, which is required to maintain turgor pressure during drought stress. Calcium plays a vital role in the formation of cell walls and relations among cells, while it also acts as a regulator in the cation-anion balance and as a catalyst for some enzymes. The decline in the contents of Ca in all of the combinations was assumed to have been a result of the transpiration rate decline under circumstances such as drought stress (Maggio et al., 2007). Magnesium facilitates the interaction of enzymes and substrates by creating chelate bonds, which accelerate enzymatic processes. Additionally, it participates in the production, transport, and storage of carbohydrate, protein, and fat molecules. Magnesium's primary purpose, however, is in the creation of chlorophyll, and hence in the absorption of the light energy necessary for photosynthesis (Hlisnikovskýet al., 2019; Kostrzewska et al., 2019). Micronutrients are vital for the growth of plants and they play roles in almost all metabolic and cellular processes, including energy metabolism, both primary and secondary metabolism, cellular defense, regulation of genes, processing of hormones, and transduction and reproduction of signals, in addition to being a catalyst in many antioxidant enzymes (Shahverd et al., 2020). It has been shown that drought stress not only impacts the availability of micronutrients, particularly those with a slow diffusion rate, but also their competitive absorption and transport. These alterations are seen as adaptive reactions to drought (Armada et al., 2014). Grafting the drought-sensitive "Josefina" tomato scion onto the "Zarina" rootstock improved the accumulation of macro and micronutrients. (Sánchez-Rodríguez et al., 2014). Several studies in the literature have reported that specific grafting combinations were more significantly efficient with regards to the absorption and transportation of nutrients like N, K, Ca, Fe, and other micronutrients, to the shoots when compared with ungrafted plants (Al-Juthery et al., 2019).

Reduced chlorophyll concentration restricts plant development, most likely as a result of ROS-induced chlorosis, photo-reduction, and triplet chlorophyll synthesis, which cause significant damage to photosystems I and II and chlorophyll generation in plants (Singh et al., 2018). Under drought stress, decreasing chlorophyll concentrations were detected in both grafted and ungrafted plants, but grafted salt-tolerant plants, notably SCP-2/TLR-2, exhibited high chlorophyll content (Figure 2). These results indicated that grafting mitigated the inhibition of photosynthesis that occurred due to drought stress. Carotenoids play a crucial role in light harvesting and oxidative damage protection through the deactivation of $_1O^2$, satisfying the excited triplet state in chlorophyll, and the enhancement of carotenoid synthesis as a means of protecting itself from photo-damage caused by cell division arrest when stressed (Singh et al., 2018). Under drought stress, the chlorophyll content of grafted tomato plants was considerably higher than that of ungrafted tomato plants, according to a tomato research (Sánchez-Rodríguez et al., 2012).

A biomarker used for oxidative stress-induced lipid peroxidation is MDA content measurement or quantification (Kaushal, 2019). During stress, the main targets of ROS are membrane phospholipids that contain polyunsaturated fatty acids. The results of this include the degradation of fatty acids and lipid peroxidation, which generate a number of cytotoxic products like MDA. In the current study, the lipid peroxidation of the melon plants increased under drought stress. It was noted, however, that the grafted plants exhibited decreased MDA contents under drought stress when compared to the ungrafted salt-stressed plants, which suggested lower ROS accumulation and less damage to the membranes of the grafted plants (Figure 2). This increased effect on the accumulation of MDA could have been related to the higher accumulation of secondary metabolites, resulting in better stabilized subcellular structures, such as membranes and proteins, scavenge-free radicals, and cellular redox buffering potential under stress (Islam et al., 2016). Reducing the MDA content in grafted, stressed plants can be an effective mechanism for mitigating the activation of plant defenses. This process also guarantees membrane integrity and reduces the leakage of essential ions (Penella et al., 2016). Ulas et al. (2020) confirmed similar results, stating that our findings clearly suggest that grafting with *Cucurbita maxima* and *Cucurbita moschata* rootstocks significantly influenced the biochemical responses of the scions (melon) under both control and salt stress conditions. ROS are produced as a result of the oxidative stress caused by, in this study, drought. High concentrations of ROS damage DNA, proteins, carbohydrates, and lipids, and hence their concentrations in plant cells must be controlled. ROS elimination, damage management, and repair can be controlled by detoxification signaling. In melon, biochemical properties, such as chlorophyll, MDA content, and antioxidant enzyme activities, such as catalase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase, were shown to increase under drought stress when compared with self-grafted and ungrafted plants (Fu et al., 2018). Under drought stress, the catalase, superoxide dismutase, ascorbate peroxidase, and glutathione reductase activities of the grafted plants SCP-1/TLR-2, SCP-1/Albatros, SCP-2/TLR-2, and SCP-2/Albatros were much greater than those of the other combinations. The improved antioxidant system activity in grafted plants resulted in a considerable drop in MDA concentrations as compared to ungrafted plants (Figure 3), indicating reduced oxidative stress. This indicated that the antioxidant enzyme mechanism functioned successfully in the grafted plants to scavenge excess H_2O_2, hence protecting the plants from ROS-induced toxicity under drought stress. The salt/drought-tolerant grafted plants had an increased resistance to drought stress as a result of their enhanced antioxidant system, which resulted in increased plant growth. By grafting onto a drought-tolerant rootstock, it is possible to improve scion performance by regulating the antioxidant system during drought stress (Liu et al., 2014).

Conclusion

Significant variations in the physiological and biochemical responses of the grafted plant combinations under drought stress were discovered in the current investigation. The increased drought resistance in grafted plants was due to the selection of salt/drought-tolerant rootstock, since the scions grafted onto the various rootstocks behaved differently to water stress. Moreover, higher levels of macro and micro element uptake were determined with the use of tolerant rootstocks under drought stress conditions. An efficient antioxidant system capable of mitigating oxidative damage is critical for increasing drought tolerance in grafted plants. The current study's findings indicate that grafting onto salt/drought-tolerant rootstocks may be an efficient, effective, and ecologically friendly approach for avoiding or minimizing production losses due to drought.

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CHAPTER 4

ECONOMIC VALUATION OF BIODIVERSITY AND AN ASSESSMENT FOR TURKEY

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1. Introduction

Biological diversity has a long history of usage, but it was first used as a concept in scientific studies published in the 1980s. In one of these studies, biological diversity included two concepts: genetic diversity and ecological diversity. In the other study, biological diversity was considered at three levels; genetic diversity, species diversity and ecological diversity. The contracted form of biological diversity (biodiversity) was used by Walter G. Rosen in 1985 (Harper and Hawksworth, 1994). The concept of biodiversity became widespread in the international arena with the "Convention on Biological Diversity", which was accepted at the United Nations Conference on Environment and Development (UNCED) held in Rio de Janeiro in 1992 and entered into force in 1993. In this Convention, biodiversity has been defined as "*the variability among living organisms from all sources including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems*" (UN, 1992).

Biodiversity is classified into four levels as genetic, species, ecosystem and functional diversity. At the most basic level, genetic diversity refers to the degree of variation in the genetic structure of individuals within a species. Species diversity, on the other hand, refers to the variety and abundance of different types of organisms in a particular habitat. The ecosystem diversity in the third stage also refers to the diversity of habitats in a particular region, that is, natural habitats. Functional diversity, on the other hand, is the result of the diversity of the first three stages and can be expressed as the diversity of complex relationships that arise in the interaction of organisms with each other and with the physical environment. In other words, functional diversity is the result of interactions between the structure and processes of ecosystems (Nunes et al., 2001a; Cho et al., 2008; Demir, 2009a).

As stated in the Convention on Biological Diversity, "*the contracting countries are conscious of the intrinsic value of biological diversity and of the ecological, genetic, social, economic, scientific, educational, cultural, recreational and aesthetic values of biological diversity and its components, are concerned that biological diversity is being significantly reduced by certain human activities and noting that it is vital to anticipate, prevent and attack the causes of significant reduction or loss of biological diversity at source*". Also, in article 11 of the convention, it is stated that "*Each Contracting Party shall, as far as possible and as appropriate, adopt economically and socially sound measures that act as incentives for the conservation and sustainable use of components of biological diversity.*" (UN, 1992).

In addition, at the 4th Conference of the Parties held in 1998 within the scope of the Convention on Biological Diversity, the statements that "*rec-*

ognizing that economic valuation of biodiversity and biological resources is an important tool for well-targeted and calibrated economic incentive measures" and "*encourages Parties, Governments and relevant organizations to take into account economic, social, cultural and ethical valuation in the development of relevant incentive measures*" emphasize that it is important to determine economic value of biodiversity (UN, 1998). At this point, we can say that in order to develop biodiversity policies, protect biodiversity and ensure sustainable biodiversity, it is vital to estimate the economic value of biodiversity.

In this context, in this study it is aimed to discuss how the economic value of biodiversity is determined. For this purpose, firstly what ecosystem services are and how these ecosystem services are valued is mentioned. Then, by emphasizing the importance of determining the economic value of biodiversity, the methods used in valuation and the advantages and weaknesses of these methods were examined. Finally, studies on the economic valuation of biodiversity in Turkey were evaluated.

2. Economic Valuation of Ecosystem Services

Different ecosystems such as forest, agricultural land, coastal and mountain ecosystems, provide many goods, services and benefits that are essential for all living things. Within the scope of an international program (Millennium Ecosystem Assessment - MEA), which was put into practice under the auspices of the United Nations in 2001, the impact of changes in ecosystem services on people's well-being was tried to be determined. In this program, ecosystem services are categorized as provisioning services, supporting services, regulating services and cultural services. Table 1 shows the ecosystem types around the world and the ecosystem services that each provides (MEA, 2003; Pagiola et al., 2004). Biodiversity emerges as one of the most important ecosystem services provided by all ecosystem types.

Table 1. Main ecosystem types and provided ecosystem services

Ecosystem services	Types of Ecosystems									
	Forest	Cultivated	Dryland	Urban	Inland water	Coastal	Marine	Polar	Mountain	Island
Freshwater	+				+	+		+	+	
Food	+	+	+	+	+	+	+	+	+	+
Timber, fuel, and fiber	+	+				+				
Biodiversity regulation	+	+	+	+	+	+	+	+	+	+
Nutrient cycling	+	+	+		+	+	+			
Air quality and climate	+	+	+	+	+	+	+	+	+	+
Human health	+		+	+	+	+				
Natural hazard regulation	+				+	+			+	
Cultural and amenity	+	+	+	+	+	+	+	+	+	+
Novel products	+	+	+		+		+			
Detoxification	+		+	+	+	+	+			

All these goods and services provided by different ecosystems, in other words by natural resources, were previously assumed to be almost unlimited; defined as "common goods" or "free goods". This approach was dominant in the industrialization policies until the 1960s and so natural resources in the world considered as sufficient for all countries to industrialize and grow their industries (Karabıçak and Armağan, 2004). Seeing natural resources as free goods led to excessive and unsustainable consumption of these resources. In the 1970s, it was understood that the economic development, which irreversibly destroyed the natural resources, which is the most important source of wealth for humanity, could not be sustainable in the long run. For this reason, an approach based on human and environmental dimensions in economic development has begun to be adopted and the concept of sustainable development has come to the fore (Kaynak, 2009).

As a result, with the evaluation of natural resources as scarce resources, the idea that these resources should have a certain price in return for providing a benefit by using them by consumers or the value of the benefit obtained has emerged. While it is easier to determine the value of goods and services that have a direct market, since the price is formed in the market, it is difficult to estimate the values of environmental goods and services that do not have a market. For this reason, various approaches and methods have been developed for economic valuation of all ecosystem services mentioned above.

The concept of total economic value (TEV), which is one of these approaches, is expressed as the sum of the economic values of all goods and services that any environmental asset or natural resource may have (Türker, 2008). TED has been widely used, especially in recent years, to quantify all the values of different components of natural resources (Merlo and Croitoru, 2005). The components that make up TED are classified in different ways. According to the generally accepted classification, TED is divided into two main headings as use values and non-use values (Merlo and Croitoru, 2005; Plottu and Plottu, 2007).

Use values are benefits derived from actual use of resources and divided into three as direct use value, indirect use value and option value. On the other hand, non-use values are benefits not linked to the actual use of resources and these values are consist of existence value and bequest value (Figure 1). Option value can take place under the title of use value (Pearce and Moran, 1994) or under the title of non-use values (Hodge, 1994) according to different approaches.

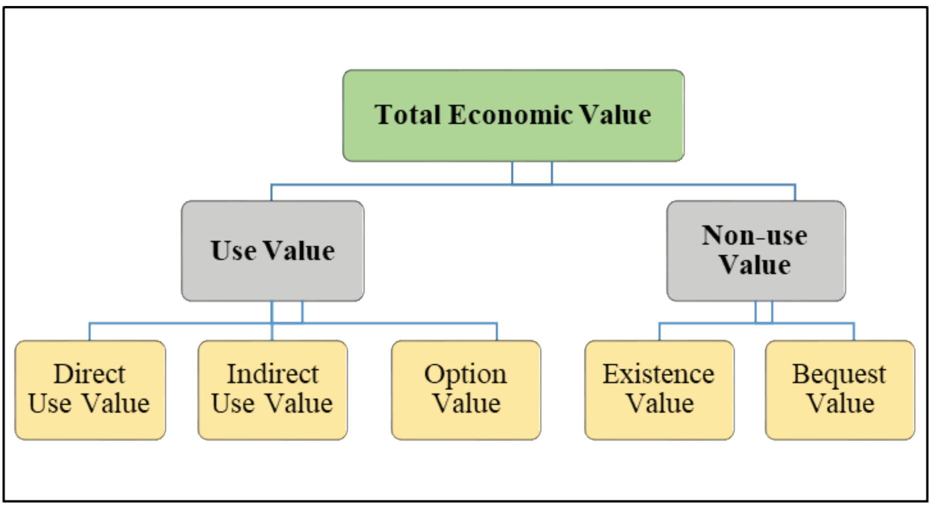

Figure 1. The Components of Total Economic Value

Various valuation methods have been developed to determine/estimate the total economic value of ecosystem services. Valuation methods can be classified in different ways based on their different characteristics. For example; methods are classified as (i) direct market value approach, (ii) revealed preferences approach and (iii) stated preferences approach (TEEB, 2010). In another classification, it is classified as follows, depending on whether the good or service has a market price or not (Merlo and Croitoru, 2005):

A. Market price available

i. Efficient price

ii. Shadow price

B. No market price available

i. Demand curve approaches: revealed preferences and stated preferences

ii. Non-demand curve approaches: Cost-based methods

3. Economic Value of Biodiversity

The relationship between the components of biodiversity, such as genes, species, ecosystems and functional diversity, plays an important role in the formation, protection and continuity of ecological balance. Biodiversity contributes to the provision of many ecosystem goods and services, such as food, nutrient cycling, climate regulation, soil formation, erosion prevention, tourism, aesthetic and cultural values. In this way, biodiversity has an important role in economic processes as well as being an important part of ecological processes. However, the destruction of biodiversity also brings ecological and economic losses that are very difficult to compensate (Demir, 2009b).

Although biodiversity provides a wide variety of direct and indirect benefits to humans, especially in recent years, human activities also cause biodiversity losses that threaten the sustainability of all ecosystems. At this point, in order to protect biodiversity in a sustainably, it is necessary to determine exactly what all the ecosystem services provided by biodiversity are and to estimate the economic value of biodiversity by using economic value determination methods.

Estimating the economic value of biodiversity is one of the most challenging issues facing environmental economists today. Economic valuation of biodiversity provides the opportunity to choose among different biodiversity policy options. In addition, the monetary valuation of biodiversity allows economists to perform environmental accounting and natural resource damage assessment. In addition, valuation is also important in terms of consumer behavior. Valuation is also used in determining consumers' views on biodiversity management goals and in identifying individual consumer motivation for biodiversity conservation (Nunes et al., 2001b).

The following benefits can also be achieved through valuation studies (OECD, 2002):

- Awareness of biodiversity can be increased.
- Land use decisions can be made according to protection or other usage purposes.
- Priorities for biodiversity conservation can be determined.
- Negative interventions to biodiversity can be limited.
- Trading of endangered species can be banned or limited.
- The effects of non-biodiversity investments on biodiversity can be evaluated.
- National economy accounts can be revised.
- Economic instruments such as taxes and subsidies can be chosen to conserve biodiversity.

When estimating the economic value of biodiversity, firstly we consider which value is desired to be calculated for biodiversity. These values are classified in different ways. For example, in a classification made by Pearce (2001) the values related to biodiversity are divided into five. These are:

- ***Direct international use values (tourism):*** Nature tourism, which has become a growing sector around the world, is based on biological resources and the richness of biodiversity. For example, wildlife viewing becomes more interesting the more species that can be observed.
- ***Direct global use values (agriculture and pharmaceuticals):*** Biodiversity contains valuable information that can be used to develop goods and services that benefit people. This information is important in matters such as the use of plants in drug production and plant breeding.
- ***Indirect global use values (ecosystem resilience):*** Since biodiversity is of critical importance for the continuation of ecosystem functions,

a decrease in biodiversity may endanger the survival of the ecosystem. Therefore, the value of biodiversity can be estimated based on the value of ecosystem functions.

- ***Indirect global use values (ecosystem services):*** Ecosystems provide many services such as watershed protection, climate regulation and nutrient cycling. Many of the benefits of ecosystem services accrued locally, as all living organisms depend on these functions, these local benefits contribute to an overall global benefit.

- ***Non-use values:*** These are the existence and bequest values of biodiversity. In order to estimate the non-use values of biodiversity, the willingness to pay of individuals are tried to be estimated using value determination methods.

In another study, different values of biodiversity were classified as follows (Nunes et al., 2001a):

- ***Instrumental value - intrinsic value:*** While there are those who argue that biodiversity has an instrumental value for a purpose, that is, its value expressed in monetary terms, many people argue that it is wrong to attribute an instrumental value to biodiversity, and that biodiversity has a unique value, that is, an intrinsic value.

- ***Monetary indicators - biological indicators:*** The monetary value of biodiversity depends on its impact on human welfare. Determining the economic value of biodiversity leads to monetary indicators and it is possible to compare and rank alternative biodiversity management policies. On the other hand, there are those who argue that the value of biodiversity should also be evaluated with non-monetary indicators such as species and ecosystem richness indices.

- ***Direct value - indirect value:*** While direct value refers to people's use of biodiversity in terms of production and consumption, indirect value is associated with a minimum level of ecosystem infrastructure, without which there would not be the goods and services provided by it.

- ***Biodiversity - biological resources:*** In economic valuation studies, the concepts of biodiversity and biological resources are often intertwined, and in these studies, economic valuation of 'biodiversity' is actually about the value of biological resources and it is confused with the value of diversity. For this reason, we pay attention to the distinction between concepts in valuation studies.

➢ ***Value of levels – changes of biodiversity:*** Economists argue that valuation should focus on change in biodiversity rather than different levels of biodiversity, while non-economist researchers aim to determine the value of different levels of biodiversity.

➢ ***Local diversity - global diversity:*** Although loss of biodiversity is generally considered a global problem, valuation studies in biodiversity generally address policy changes or different scenarios at local, regional or national level. Although this seems contradicting, it can be argued that biodiversity and its loss are relevant at multiple spatial levels, from local to global.

➢ ***Genetic value – other life organization levels:*** While natural scientists determine the value of biodiversity, they focus on the genetic or species levels, while social scientists consider the value of biodiversity at the species and ecosystem levels.

➢ ***Holistic approach - reductionist approach:*** According to those who adopt the holistic approach, biodiversity is an abstract notion, linked to the integrity, stability and resilience of complex systems, and thus difficult to disentangle and measure. On the other hand, those who adopt the reductionist approach argue that it is possible to divide the total value of biodiversity into different economic value categories (such as direct use and non-use value).

➢ ***Expert assessment - general public assessment:*** According to some people, valuation should only be done by experts. Those who support the other view state that the general public, which includes individuals with different education levels and life experiences, should also take part in valuation studies.

As it can be understood from the aforementioned approaches, it is difficult to talk about a single correct method or approach when estimating the value of biodiversity. The point to be decided here is which point of view should be adopted. In general, during economic valuation of biodiversity, its instrumental value is tried to be determined. In other words, the value of biodiversity is determined as the value that emerges as a result of the interaction between human and biodiversity. In this context, it is possible to estimate the economic value of biodiversity with the total economic value approach used to calculate the economic values of all goods and services that ecosystem resources may have.

3.1. Economic Valuation Methods

As in ecosystem services, while estimating the economic value of biodiversity, firstly we look at whether there is a market for the good or service offered. If the good or service has a market, the direct market price of that good or service or its shadow price is used in economic valuation. In goods and services that do not have a market, various valuation methods are used, which are discussed under two main headings as demand curve approaches and non-demand curve approaches.

As some of the benefits of biodiversity have a market price, this price is used for economic valuation of the benefits. For instance, financial revenues from tourism activities related to biodiversity and the value of contracts signed between the pharmaceutical industry and government institutions are evaluated within this scope. However, since there is no direct market for many of the benefits that biodiversity provides, various valuation methods based on consumer preferences are utilized. The classification of these methods is shown in Table 2. The methods with the demand curve approach are included in two subheadings, namely the stated preference methods and the revealed preference methods (Kaya, 2002; Merlo and Croitoru, 2005; Gürlük, 2006; Deniz, 2012).

Table 2. Methods used for economic valuation of non-market goods and services

Demand Curve Approach		Non-Demand Curve Approaches
Stated Preference Methods	Contingent Valuation Method	
	Choice Modelling	
Revealed Preference Methods	Travel Cost Method	Damage Avoided Cost Method
	Hedonic Pricing Method	Replacement Cost Method
	Hedonic Travel Cost Method	Preventive Expenditure Method
	Production Function Method	Opportunity Cost Method

3.1.1. Demand Curve Approaches

In this approach, the economic values of the benefits provided by environmental goods and services can be determined directly or indirectly. Direct demand curve methods aim to reveal individuals' willingness to pay and willingness to accept values by directly questioning the value proposals of individuals. These methods are also called "stated preference methods". Contingent valuation and choice modeling methods are under this approach (Kaya, 2002; Gürlük, 2006).

Indirect demand curve methods, on the other hand, take consumer surplus into account. These methods use market price of substitute goods and services of non-market goods and services as a proxy value. So these methods are also called "revealed preferences approach". Travel cost method, hedonic pricing method, hedonic travel cost method and production function method are included in this class (Kaya, 2002; Merlo and Croitoru, 2005; Gürlük, 2006).

A. Stated Preference Methods: The purpose of these methods is to estimate the value of non-market goods and services by asking theoretical questions. In this context, willingness to pay and willingness to accept values of people are determined (Pearce and Özdemiroğlu, 2002). In these methods, individuals are asked to rate, rank or make choice of predefined alternatives given in the theoretical scenarios (Boxall et al., 1996; Engo, 2010).

Contingent Valuation Method (CVM): This method is frequently used for valuation of all kinds of ecosystem and environmental goods and services. Although CVM is used to estimate both use and non-use values, it is widely used to estimate the economic value of non-use value components (King and Mazzotta, 2000a). In CVM, the economic value is determined by revealing people's preferences about the relevant good or service through survey questions. By presenting a hypothetical market for an environmental good or service, people's willingness to pay to obtain the benefit in question or their willingness to accept to prevent a loss of benefit is determined (OECD, 2002; Engo, 2010).

Choice Modeling Methods: It is a method based on the hypothetical scenario like CVM and can be used to determine the economic value of almost all ecosystem and environmental goods and services. Unlike CVM, it does not ask individuals to directly express their payment willingness in monetary terms. In choice modeling methods, individuals are asked to choose among alternative scenarios that include different combinations of benefits. Environmental benefit combinations with different price levels are presented to individuals in hypothetical scenarios. In this way, economic values emerge as a result of choices or rankings made by individuals (Abila et al., 2005).

B. Revealed Preference Methods: These are indirect methods that use the consumer surplus to determine the economic value of goods and services that do not have a market. In these methods, the economic value is estimated by using the existing markets of the goods or services as a proxy market (Mundial, 2004; Deniz, 2012).

Travel Cost Method (TCM): It is generally used to estimate the economic value of areas used for recreational purposes. The time and travel cost expenses that people incur to visit an area can be handled as price of access to the area. Thus, people's willingness to pay to visit the area is estimated based on the number of visits at different travel costs level (King and Mazzotta, 2000b). The time and travel cost expenses of the visitors are determined through survey studies conducted with the visitors in the recreation areas.

Hedonic Pricing Method: The method considers a product or service with a market as a 'bundle of characteristics', each with its own implicit price, some of which may be non-market in nature. By determining the relationship between the qualities that do not have a market and have an implicit price and the market price of the product or service, the economic value of that quality that does not have a market is tried to be estimated (Kaya, 2002; OECD, 2002). But this method is rarely used in addressing the value of biological resources, as it is difficult to describe biodiversity as a part of bundle of attributes.

Production Function Method: The method emphasizes that "improvements in resources or environmental quality as a result of improved ecosystem services reduce the costs and prices of marketed products and increase their quality" (TEEB, 2010). The method assumes that environmental goods and services function as factors of production in the production process of products. Thus, the change in the availability of environmental goods and services affects the cost of marketed products and the availability of supply (NRC, 2004).

3.1.2. Non-Demand Curve Approaches

Some economic valuation methods use current expenditures, market prices, or the costs to be incurred in the production or absence of the product or service, instead of consumer surplus. These are:

Damage Avoided Cost Method: It aims to estimate the value of ecosystem services based on the cost incurred to avoid damage from loss of ecosystem services. This method uses either the value of the protected property or the cost of actions taken to avoid damages, as a measure of the benefits provided by an ecosystem (Abila et al., 2005).

Replacement Cost Method: This method estimates economic values based on costs of replacing ecosystem services that are destroyed or damaged, or costs of restoring the ecosystem so that it again provides the service. The method uses the cost of replacing an ecosystem or its services as an estimate of the value of the ecosystem or its services. This method is generally used to calculate the cost of pollution on environmental resources (Dixon and Pagiola, 1998; Abila et al., 2005).

Preventive Expenditure Method: This method aims to determine the cost or benefit value of environmental resources by trying to observe how much individuals, communities or society will spend to prevent environmental damage. In doing so, the benefits of environmental resources are estimated indirectly (Abila et al., 2005; Engo, 2010).

Opportunity Cost Method: In this method, it is accepted that the value of the good or service is at least equal to the value of the best alternative abandoned. The value of non-market good or service is determined according to the value of the opportunities given up to produce this good or service (Kaya, 2002). For example, the value of a protected area is also calculated based on the revenue from another best alternative use of that area (Engo, 2010).

Benefit Transfer Method: This method is not a valuation method and it makes use of the economic value estimations obtained from previous similar studies and adapts these values to the current study area, and accordingly, the economic value for the existing area is estimated. In other words, it is to use the value of the benefit provided by one environmental resource in estimating the value of another resource. The method is generally preferred when it is too expensive or not enough time to perform valuation studies (Plan Bleu, 2015).

Table 3 shows the use cases of the above-mentioned valuation methods in estimating the economic value of different biodiversity value components, as well as the advantages and weaknesses of the aforementioned methods. In estimating the economic value of biodiversity, the contingent valuation method emerges as the most preferred method, which can be used for all value components and ecosystem services.

Table 3. Comparison of economic valuation methods

Method	**TEV categories**	**Related biodiversity components**	**Advantages of the method**	**Limitations of the method**
Market price	Direct and indirect use values	Products with a market (agricultural products, medicinal plants, tourism and recreation)	Include easily available and real market data	Can only be used for goods and services that have a market

Contingent Valuation	Use and non-use values	Conservation of genetic and species diversity, Habitat protection, All ecosystem functions related to biodiversity, Bequest and existence values of biodiversity	Used in estimation of all use and non-use values	Based on hypothetical market and scenarios, Many sources of bias
Choice modelling	Use and non-use values	Conservation of genetic and species diversity, Habitat protection, All ecosystem functions related to biodiversity, Bequest and existence values of biodiversity	Used in estimation of all use and non-use values	Based on hypothetical market and scenarios, Many sources of bias
Travel cost	Direct and indirect use values	Recreational activities	Based on real market data	Difficult to estimate the value in visits to more than one place, The need for more data
Hedonic Pricing	Direct and indirect use values	In determining the effect of biodiversity on housing prices	Based on real market data	Very difficult to determine the exact effect of the biodiversity component on housing prices, The need for more data on the housing market
Production Funciton	Indirect use values	Biological resources that are production inputs for goods that have a market	Include easily available and real market data	Need for extra data and difficult to determine the impact of changes in services on production process
Cost based methods	Direct and indirect use values	All ecosystem functions related to biodiversity	Include easily available and real market data	The true value can be overestimated, Costs also have secondary benefits and they are not calculated

Benefit transfer	Use and non-use values	All ecosystem functions related to biodiversity	Less costly, Easy to apply	Failure to give reliable results when the properties of the application areas are not the same

3.2. Studies About Economic Valuation of Biodiversity in Turkey

Turkey has three biogeographic regions, called Europe-Siberia, the Mediterranean and Iran-Turan, and the transition zones of these regions, and has three different bioclimatic types. Also due to topographic, geological, geomorphological and soil diversity, altitude differences ranging from 0-5000 meters and having various ecosystem types, Turkey has gained a feature of a small continent in terms of biodiversity. Turkey has agricultural, forest, mountain, steppe, wetland, coastal and marine ecosystems and different forms of these ecosystems and habitat diversity. For this reason, it is very rich in terms of both flora and fauna and is important in terms of genetic diversity (MoEF, 2008; MoAF, 2019).

The Rio Summit, which was held in 1992, was a turning point in the conservation and sustainable use of biodiversity in our country as well as in the world. At this summit, global issues such as biodiversity, forest resources and climate change were discussed and solutions were sought at the global level. As a result of the summit, important agreements such as the Convention on Biological Diversity, Framework Convention on Climate Change and the Forest Principles were signed by the countries. Turkey signed the Convention on Biological Diversity, one of these conventions, in 1992 and ratified it with the Law No. 4177 dated 29 August 1996. The Convention entered into force in Turkey on May 14, 1997 (MoEF, 2008).

Following this historical summit, awareness of natural resources such as biodiversity and forest resources has increased in Turkey as well as in the world, and in this context, studies on issues such as the protection of natural resources, their sustainable use and the determination of their economic values have gained importance. Within the scope of the Convention on Biological Diversity, it is envisaged that each country will determine its own priorities in order to achieve the objectives set out in the Convention and that national biodiversity strategy and action plans will be prepared in order to implement the adopted work programs.

Turkey's National Biological Diversity Strategy and Action Plan

(NBSAP) was prepared by the Ministry of Environment. Then, due to the changing national and international conditions and trends, the aforementioned action plan was updated in 2007 with a participatory process within the scope of the "Convention on Biological Diversity Implementation Project" carried out with the grant support of the United Nations Environment Program (UNEP) and the Global Environment Fund (GEF). In this action plan, the aims and targets for the protection of biological diversity in Turkey and the action proposals necessary to reach these targets are included (MoEF, 2008).

Although, economic valuation of biodiversity is encouraged within the scope of the Convention on Biological Diversity, there are no targets or statement about economic valuation of biodiversity in the aims and strategies of the above-mentioned action plans.

Again, at the 10th Conference of the Parties of the Convention on Biological Diversity held in 2010, it was decided to review, update and revise the NBSAPs of the party countries until 2014. In line with this decision, various meetings and workshops were held in our country in the following years, and as a result, the National Biological Diversity Action Plan (2018-2028) was accepted. This is the current action plan on biodiversity in our country. In this plan, 7 national objectives and related actions were determined (MoAF, 2019).

When the above-mentioned national objectives of the National Biological Diversity Action Plan and the actions determined for the realization of these targets are examined, as in the NBSAP, no action has been determined regarding economic valuation of biodiversity. On the other hand, within the scope of the NBSAP, which is in force for the period of 2007-2017, various comprehensive projects have been carried out in our country with the support of related public institutions, organizations and international organizations.

In some of these projects, the economic value of biodiversity was also tried to be estimated. For example, within the scope of the "Enhancing the Management of Forest Protected Areas Project", which is one of these projects and carried out by the Ministry of Environment and Forestry, GEF and the United Nations Development Program (UNDP). A pioneer research has realized for Küre Mountains National Park, and the economic value was tried to be estimated. In this context, the ecosystem values of the area, the benefits obtained from these services and the economic values of the benefits have been determined (Lise, 2011).

In a similar study, the value of ecosystem goods and services, including biodiversity, of forest resources in the Yıldız Mountains was tried to be estimated within the scope of the "Conservation and Sustainable Development of Biological Diversity and Natural Resources in Yıldız Mountains Project". In the study, the value of related goods and services was estimated by using the benefit transfer method (YMBP, 2010).

Within the scope of the "Training of Interest Groups and Creating Guidelines for the Integration of Nature Conservation into the Economic System" project carried out by the Ministry of Forestry and Water Affairs, an attempt was made to evaluate (bioassess) the ecosystem services provided by Sultan Reedbed National Park. In this study, bioassessment of goods, services and benefits such as agricultural assets, flora, fauna, organic matter and pollination of Sultan Reedbed National Park was tried to be realized (OSİB, 2012).

In addition, the value of forest resources was tried to be estimated by the experts of the World Bank, in the example of Bolu Regional Directorate of Forestry, within the scope of the "World Bank's Turkey Environment and Natural Resources Technical Assistance Program". In this study, by using different valuation methods; direct use, indirect use, option and non-use values have been tried to be estimated. The values of biodiversity, medicinal plants and watershed protection were estimated due to benefit transfer method (World Bank Group, 2015).

In a study, carried out within the scope of the project named "Optimizing the Production of Goods and Services by Mediterranean Forest Ecosystems in a Context of Global Changes" financed by international organizations, Düzlerçamı forests within the boundaries of Antalya Regional Directorate of Forestry were selected as a sample area and the socioeconomic value of the goods and services provided by the area was tried to be estimated. Biodiversity protection, recreation and tourism and carbon sequestration values of the area were estimated in the study (Balkız, 2016).

Comprehensive studies to determine the economic values of all ecosystem value components of a particular area are less numerous (Başak, 2003; Gürlük, 2006; Gürçay, 2009; Pak et al., 2010; Demirci, 2017). In these studies, the economic values of various ecosystem goods and services, including the biodiversity of the areas, were tried to be estimated. In addition, studies were carried out to estimate economic value of biodiversity of a certain area (Gürlük, 2021); estimate conservation value of an area which is important in terms of biodiversity (Kubaş et al., 2007; Durgun, 2013), value protection of the wildlife in the area (Kaya et al., 2009) or value protection of only one plant species (Erdem, 2004; Demir, 2009a; Demir and Arısoy, 2014; Demir, 2019).

4. Conclusions

As a result of increase in awareness of biodiversity benefits and that it is very hard to compensate for the damages and losses that may occur in biodiversity, studies discussing protection and sustainable use of biodiversity are increasing. At this point, it is also important to estimate the economic value in order to emphasize the importance of biodiversity. Although when compared to other countries, economic valuation studies in our country are not at the desired level yet, but there has been a trend in recent years.

Economic valuation serves as a tool for the sustainable management of biodiversity and ensuring the conservation-use balance. However, since the valuation methods have various advantages or limitations, the debates on the reliability and validity of the methods continue. Therefore, in this study, the importance of economic valuation of biodiversity is emphasized and the valuation methods and advantages and weaknesses of these methods are discussed. While estimating the economic value of biodiversity, it is important to decide accurately (i) the level (genetic, species, ecosystem) of biodiversity, (ii) the value component and (iii) the economic valuation method. Based on the purpose of the study, it is necessary to try to minimize the sources of bias that will arise at these stages.

Economic valuation studies in biodiversity do not give us precise and accurate monetary results. Here, only a value estimation can be made depending on the method used. However, revealing these value estimates contributes to the increase of awareness of biodiversity by the society and decision makers, the conservation and sustainable use of these resources. At this point, it will be beneficial to make reference to value estimation studies in development plans, strategic plans and action plans where macro goals related to biodiversity are determined, and even to have incentives for valuation studies.

Although both the preparation of the National Biodiversity Action Plan and the fact that biodiversity inventory studies are carried out are positive steps in our country, it is necessary to generalize valuation studies. The dissemination of some important projects and pilot studies prepared in cooperation with various public institutions and international organizations, and the use of different valuation methods for various biodiversity value components in different project areas will help to obtain more realistic and beneficial results. Here again, it is necessary to get help from international organizations and experts, and these studies should be carried out in the coordination of the relevant public institutions in our country. In fact, it would be beneficial to record the data obtained from these studies throughout the country and to establish national standards for data collection and valuation stages.

Integrating the results of economic valuation studies in management plans, especially in protected areas, will make it easier for decision makers to make the right choice among different options and to manage the area more effectively and efficiently. In addition, valuation methods should be applied to reveal any damage that may occur in biodiversity and accordingly to calculate compensation. At this point, the non-use values of biodiversity should be estimated and included in the compensation calculations.

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CHAPTER 5

DEVELOPMENT AND CURRENT STATUS OF PROTECTED AREAS IN THE WORLD AND IN TURKEY

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1.1. Introduction

In the past, forests were used only for the production of wood raw materials, but today, as a result of the increase in social awareness, the services provided by forests have gained great importance. In addition, other non-wood products and protective services provided by forests for public health and order are of great importance in terms of the understanding of intensive forestry, which aims to meet the demands of modern society (Güngör et. al., 2018). Protected areas with a changing and developing forestry understanding have become an indispensable element of nature conservation efforts as a result of long years of knowledge and experience. The emergence of the protected area idea can be grouped under two main headings. The first is the natural-cultural values contained in the protected areas and the obligation to protect these values; the other is that people create a threat from their use of these areas and that a limitation should be imposed on these uses. In addition, protected areas play a vital role in the continuation of ecological relations and processes in the world by ensuring the protection of risk and endangered species and ecosystems, biodiversity (Kuvan, 2005). Protected areas fulfill the mitigation function by preventing the carbon loss already present in plants and soil and by keeping more carbon dioxide in the atmosphere in natural ecosystems. Protected areas are effective in maintaining the integrity of the ecosystem, regulating the local climate, reducing the risks and impacts of major natural events such as storms, droughts and sea level rise. Protected areas also fulfill an adaptive function by contributing to the maintenance of important ecosystem services that will help people cope with changes in water resources, fisheries, diseases and agricultural production caused by climate change (Dudley, N., et. al., 2010).

In addition to the ecological benefit of protected areas, the economic benefit is also extremely important. With the increase in the interest of the society in nature tourism, the demand for the recreational use of forests has also increased. This situation has presented a new source of income to the forestry industry under the name of ecotourism. Ecotourism is generally thought to be done in protected areas and is carried out with two approaches. The first understanding includes activities such as trekking in forests and protected areas, camping areas. The second understanding is that the forest areas are partially or completely opened to tourism. In this second understanding, there are activities such as allowing accommodation (pension, hotel, motel, etc.) in the forest ecosystem, picnicking in forest areas, and organizing safari tours. Although this understanding is thought to harm the forest ecosystem, there are studies that it contributes significantly to the country's economy.

1.2. Protected Area

Forests are one of the most affected areas among natural resources. The first examples of protected areas; Water resources, which are the source of life for people, are religious places, wild animal species and habitats. (Kurdoğlu, 2007).

Conservation of natural areas is a method applied to ensure that many ecological, cultural, social, etc. riches in the world are preserved and passed on to future generations, and protected areas are conceptually the application of nature protection thought. (Şen and Buğday, 2015). The idea of a protected area was first introduced at the International Flora and Fauna Conservation conference held in London in 1933. This convention is the first to be signed for the protection of endangered or rare species. The International Union for Conservation of Nature (IUPN) was established for protected areas in 1948. This organization was renamed IUCN in 1958 by the United Nations Educational, Scientific and Cultural Organization (UNESCO), an organ of the United Nations, and in 1990 this organization was named "World Conservation Union". Protected area according to the IUCN; It is defined as the legally managed land and sea areas that serve especially for the protection and continuity of biological diversity, natural and cultural resources. (IUCN, 1994).

Protected areas have been defined in different ways by international and national organizations, laws and regulations. Protected area according to the Convention on Biological Diversity realized in Rio in 1992; It is defined as geographical areas classified and managed according to defined protection purposes.

Yücel and Babuş (2005) defined protected areas as parts of nature that are necessary for the continuity of both nature and human life by protecting the plant and animal existence and habitats of nature within certain criteria. Kuvan (1999) stated that protected areas are related to national conservation and development policies and are of vital importance not only for the protection of biological diversity, but also for ensuring sustainability in terms of meeting socioeconomic needs in accordance with their resource qualities.

In addition to the environmental and ecological functions of protected areas, their social and economic functions are also of great importance. Nowadays, in many parts of the world, people continue their lives intertwined with protected areas. People directly benefit from the functions of protected areas, such as protecting soil and water and containing medicinal plants (Yıldırım and Erol, 2012). In addition, the function of protected areas is not only to realize the purpose of protecting nature, but also to provide ecological, economic and social benefits, where the recreational needs of the society can be met, without being contrary to the purpose of nature protection (Kuvan, 1999).

Protected areas are a strong and widespread status accepted in the world, as they play an important role in protecting biological diversity, which includes natural and cultural resources of international importance, and in transmitting it to future generations. Countries not only make legal arrangements within themselves, but also take measures to protect resources, ensure their continuity and operate resources according to their purposes by making international agreements (İlter and Ok, 2012).

Protected areas where human activities were prohibited in the past, only prohibitions and the logic of protection cannot prevent the intense pressures on the ecosystem. Therefore, it cannot provide effective results in the protection of natural and cultural resources (Alkan et. al., 2009). In addition, these prohibitions negatively affect the communication between the forestry organization and the society, while causing conflicts with the local people (Toksoy et. al., 2008; Toksoy and Bayramoğlu, 2017). Nowaday, it aims to protect protected areas and biodiversity, sustainability of natural resources and integration with social development processes. (Yücel and Babuş, 2005). With this changing understanding, the modern protected area approach was developed in Austria, Canada, and the USA in the 19th century, while it spread to Europe and other countries only in the 20th century. (Geray and Akesen, 2001).

When the historical process of protected areas is examined, it is seen that an understanding has emerged that the resource values of forests are not only protected and improved, but also meet the needs and requirements of the societies. With this understanding, protected areas have nine purposes. These; scientific research, wildlife conservation, conservation of species and genetic diversity, sustainability of environmental services, protection of natural and cultural values, recreation, education and training, sustainable use of natural resources, and ensuring the continuity of cultural and traditional qualities. (Anonim, 2006).

1.2.1. Development and Current Status of Protected Areas in the World

B.C. despite the immaturity of nature conservation thought and the lack of use of the concept of nature protection, there are practices related to this subject. B.C. In 252, the Emperor of India Asoka issued an edict to protect forests and animals (Wright, 1996). The King of England, William-I, ordered land survey work to be done in 1084 for the purpose of protection. This study throughout England; It has been prepared with the title of "Domesday Book" to include forests, fisheries, agricultural lands, hunting reserves, and the exchange of fertile soil resources (Yücel, 1995).

With the industrial revolution, radical changes have occurred in the field of protection. This situation has caused the foundations of today's conservation understanding to be laid. With the result of the mobility that started with the understanding of protection, protection mobility has also been realized in Europe and America (Erdönmez, 1997).

Towards the end of the first industrial revolution, in 1829, the foundations for the conscious emergence of nature protection thought, nature protection studies within the legal framework, separation of protected areas, taking legal measures and conducting scientific studies on the subject were laid (Yücel and Babuş, 2005). For the first time, Drachenfels in Bonn, Germany, and part of the Virgin Forest in 1838 was reserved for conservation purposes (İnal, 1949).

In the Yellowstone region of America, people who make a living by hunting have worked to preserve the existence of animals and plants in the region and to transfer their natural beauty to future generations. In this context, the world's first national park is the "Yellowstone National Park" in America with an area of 8,670 km2. The national park, which was declared in 1872, is officially seen by the countries as the beginning of the nature protection idea in the world (Akesen, 1978).

Many countries have adopted these concepts with the emergence of the concept of nature protection and national park. The first national parks declared in chronological order after America; "Royal National Park" declared in Australia in 1879, "Banff National Park" in Canada in 1885, "Tongariro National Park" in New Zealand in 1897 and "El Chico National Park" in Mexico in 1898 (Eagles et. al., 2002).

The first example of the idea of a national park in Europe is the "Abisko National Park", which was declared in Sweden in 1909. The first National Park in Italy, declared in 1922, is "Grand-paradiso". "Mount Vitosha" declared in 1934 is the first national park in Bulgaria. "Olympos", the first national park of Greece, was declared in 1938. The first national park declared in England in 1951 is the Peak District National Park. The first example of France is the "Vanoise National Park" dated 1963 (Anonim, 2015). The first national park in Germany is the "Bavarian Forest National Park", which was declared in 1970. Until the end of the First World War, approximately 50 national parks were declared around the world. By the end of the Second World War, this number had reached approximately 350. However, the national park management approach differs in Europe and America. In the understanding of national parks in Europe, the "human-nature" relationship is kept in the foreground, allowing science and research to be carried out, and visitors are allowed to use them for touristic purposes without harming the nature, while the national park understanding in the United States is to protect natural areas by keeping them closed to visitors (Yücel and Babuş, 2005).

Many non-governmental organizations have been established and contracts have been signed between countries in order to protect the natural habitats and the creatures living in these areas in the world and to determine the purpose of use of these areas. As a result of the non-governmental organizations established and the agreements signed, an international financing mechanism was established. In addition, non-governmental organizations have tried to emphasize the purpose and importance of protected areas by holding various conferences and symposiums.

First established in 1948, the IUCN was established as a way to promote conservation worldwide. The creation of protected areas has always been seen as an important area of focus. In 1961, a new international non-governmental organization, the World Wildlife Fund (WWF), was established to mobilize public support for conservation. This organization is considered the beginning of the growing period for international protection. In the following years, there has been a lack of information about the world's protected area data system, as each country has started to keep its own records regarding protected areas. With the understanding of this situation in 1962, the World Conference on National Parks was held and a more official movement was started around the world by publishing the United Nations (UN) List of Protected Areas.

In 1963, the African College of Wildlife Management was established in Mweka, Tanzania. In 1968, the UNESCO Man and the Biosphere Program began its studies to identify biosphere reserves. As of 2020, there are 714 reserve areas in 129 countries. With the Ramsar Convention signed in 1971, 2,413 wetlands consisting of 254.5 million hectares were taken under protection with the signature of 170 countries at the end of 2020.

In 1972, new conventions affecting protected areas were approved by the UN Conference on Environment and Development, and the Nairobi-based United Nations Environment Program (UNEP) was established. At the Second World National Parks Conference held in the USA in 1972, 10 protection categories developed by IUCN and put into practice in many countries of the world were determined. These areas are; Scientific Reserves are National Parks, Nature Monuments, Nature Conservation Reserves, Landscape Protected Areas, Resource Reserves, Anthropological Reserves, Multi-Use Areas, Biosphere Reserves and World Heritage Sites. In addition, the "Red List" was developed, which includes fauna and flora species that are at the point of extinction. The training program for protected area personnel, which began in Costa Rica in 1977, continues to this day and provides trained personnel for areas in Central America (Chape et. al., 2008).

In 1978, the IUCN system of protected area categories was published for the assessment of protected area coverage worldwide. The "World Conservation Strategy" published by IUCN, WWF and UNEP in 1980 popularized the concept of sustainable development and the partnership between conservation and development. The World Protected Area Database (WDPA) was established in the UK in 1981 by the IUCN and the National Parks and Protected Areas Commission, creating the first worldwide database on protected areas. In 1982, the concept of sustainability was introduced by the World Union for Conservation of Nature (IUCN) for the first time, that it should be managed without compromising the continuity of ecosystems and organisms that people benefit from. (Bayramoğlu and Seyhan, 2019).

In 1987, the 3rd World Congress of National Parks in Bali set the goal of 10% protected area coverage of each of the world's biomes, emphasizing the importance of protected areas as a key element in national development plans. The Global Environment Facility (GEF), created in 1991 by the World Bank, UNDP, and UNEP, provided a major new intergovernmental financing mechanism for protected areas, particularly through the later negotiated Convention on Biological Diversity (CBD). In 1992, the 4th World Congress on National Parks and Protected Areas emphasized the links between protected areas and other sectors.

At the World Summit in Rio de Janeiro, Brazil, the Climate Change Framework Convention with the CBD, which has great relevance to protected areas, was ratified. The World Summit on Sustainable Development, held in Johannesburg, South Africa in 2002, called for the reversal of biodiversity loss by 2010 and the establishment of a comprehensive system of marine protected areas by 2012. With the 5th World Parks Congress in 2003, the importance of protected areas for sustainable development was re-emphasized. Finally, at the 7th meeting of the CBD Conference of the Parties in 2004, a comprehensive protected areas work program was adopted to support the implementation of CBD in situ conservation components (Chape et. al., 2008).

The World Heritage Convention was adopted in 2006. As of 2020, 213 natural World Heritage sites and 39 mixed natural and cultural sites in 167 countries cover an area of 336.87 million hectares. With the influence of environmental processes, the number of protected areas in the world is increasing day by day. According to WDPA, there are 261,216 designated protected areas on Earth. 243,767 of them are terrestrial protected areas. These areas cover an area of approximately 20 million km² in total and correspond to 15.3% of the world's land area. Although marine protected areas are less in number than land protected areas, they are 174,490 in total. These areas, which cover more than 6 million square kilometers of the world, correspond to 7.56% of the world's ocean. When the regional distribution of protected areas is examined, there are 37,184 protected areas in the Asia-Pacific region, 378 in Western Asia, 160,695 in Europe, 8,448 in Africa, 9,767 in South America, 44,678 in North America and 35 in the Poles (URL-1, 2020).

1.2.2. Development and Current Status of Protected Areas in Turkey

Turkey is a country that differs in terms of landforms and geology, and these differences constitute the diversity of flora-fauna species and resources. Turkey is divided into three phytogeographic regions; It is a flora area of Euro-Siberia, Mediterranean and Iran-Turan. The presence of three different flora areas in Turkey has enabled it to have very rich ecological reserves (Saya and Güney, 2014).

Many applications have been made about the protected areas since the Ottoman Empire. In the 15th century, grazing, agriculture and construction in the river basins were prohibited in order to prevent certain streams from filling up in Istanbul. In addition, soil erosion was tried to be prevented by planting couch grass on steep slopes. (Anonim, 2007).

In 1727, protection measures were taken by intervening in illegal cuttings in the forests in Istanbul. Again in 1767, constructions were stopped due to shortage of wood and water (Yalınkılıç and Arpa, 2005). In the 18th century, with the 'Tulip Age', a new park-garden business came to the fore in the Ottoman Empire, and the protection of nature for recreational use was indirectly ensured.

In the Ottoman Empire, with the Tanzimat Period, non-governmental organizations played a leading role in the problems related to the protection of forests and made important efforts. However, the increasing sensitivity of forest and nature protection by the society has led to significant developments. These developments;

- The Land Code, enacted in 1858,
- The Forest Regulation, which was enacted in 1870, due to the fact that the protection of forests with the Land Law was insufficient,
- Mecelle-i Ahkam-ı Adliye (Civil Law), which was completed in 1876 (Özdemir, 2002).

These developments reveal the attitude of the Ottoman Empire towards forest and nature protection.

In the last periods of the Ottoman Empire, there were important developments regarding forests. The management directive, which came into force in 1919, is one of them, and with this instruction, forests are divided into two as "operation forests" and "protection forests" (İnal, 1949).

With the establishment of the Republic of Turkey, forests continued to be managed as operational and protection forests. As a result of this management approach, the term "National Park" was used by Inan for the first time. In parallel with the developments in the field of nature protection in the world, it has been revealed that a different conservation approach is needed in Turkey. The Convention on the Regulation of Whaling, signed by Turkey in 1931, is very important in terms of being the first international convention in terms of nature protection (Yeşil, 2016).

The Forest Law No. 3116 enacted in 1937 is the first “Forest Law” of the Republic of Turkey. With this law, the definition of the forest, its property status, protection etc. were defined. Although terms such as national park and nature park are not included in the law, the protection of forests is mentioned in Chapter 5 of the Forest Law, and it is clearly stated in Chapter 8 of the Forest Law, which forests will be reserved for conservation forests. Despite this, no forest was declared as a conservation forest in Turkey until the 1950s. Istanbul Belgrad Forest, which was declared in 1951, has the characteristics of the first announced conservation forest (Teksöz et. al., 2014).

In 1956, with the 4th and 25th articles of the Forest Law No. 6831, the term "National Park" entered Turkish laws for the first time in legal terms. Yozgat Çamlık National Park became the first national park to be declared in 1958 with the enactment of the Forest Law No. 6831 and studies on the national park continued until the 1980s (Yücel, 2005).

National Parks were separated from the Forest Law No. 6831 with the National Parks Law No. 2873 enacted in 1983 and the National Parks Regulation enacted in 1986. With the National Parks Law, four different protected area statuses have been determined in Turkey. These protection statuses are "National Park, Nature Parks, Nature Monument and Nature Protection Area" (T.C. Resmi Gazete, 1983). These areas are in the law numbered 2873;

1. National Park; "In terms of scientific and aesthetics, national and international rare natural and cultural resource values and natural parts with protection, recreation and tourism areas",

2. Nature Parks; "Natural parts with vegetation and wildlife characteristics, suitable for the recreation and entertainment of the public in the integrity of the landscape",

3. Nature Monument: "parts of nature that have the characteristics of nature and natural events, have scientific value and are protected within the principles of national parks",

4. Nature Protection Area; It is defined as "the parts of nature that contain rare, endangered or extinct ecosystems, species and outstanding examples of natural events that are important in terms of science and education, and which must be strictly protected and reserved for use only for scientific

and educational purposes".

The numbers and areas of protected areas in Turkey according to the National Park Law No. 2873 are given in Table 1.

Table 1. Number and areas of protected areas in Turkey

Status of Protected Areas under the National Park Law	Number of	Area (Ha)
National Park	45	879.768
Nature Monument	116	9.389
Nature Protection Area	30	46.726
Nature Parks	247	106.836
Total	438	1.042.719

According to the National Park Law No. 2873, there are currently 438 protected areas in Turkey, including 45 National Parks, 116 Nature Monuments, 30 Nature Protection Areas, 247 Nature Parks, and a total of 1,042,719 ha is used for this purpose. These areas correspond to 1.3% of Turkey's surface area.

The first Natural Monument in Turkey, which was declared in 1988, is the Samandere Waterfall located in the Samandere village of the centre district of Düzce Province, the first Nature Conservation Area declared in 1987 is the Hacıosman Forest located in the Tekkeköy district of Samsun province. The first Nature Park declared in Turkey is Ölüdeniz Kıdrak Nature Park in the borders of Fethiye district of Muğla Province, which was determined and taken under protection in 1983.

Figure 1. Protected area map of Turkey (URL-2, 2019).

Protected areas, especially national parks, need well-prepared management plans (long-term development plan or master plan) in order to protect, develop and maintain the natural, cultural and recreational resource values. In order to ensure an effective management in protected areas, systematic evaluation studies should be carried out based on participation and taking into account scientific principles (Daşdemir and Güngör, 2008). In addition, the density of use of cities has increased due to the increasing rapid population, and accordingly, it has become necessary to take sustainable planning and design decisions to meet the demands of the modern city user, leaving the traditional arrangement understanding (Bayramoğlu and Yurdakul, 2019).

Result

There are many problems related to protected areas, such as legal gaps, authority confusion, financial inadequacies, deficiencies in the planning and control process, problems arising from intense touristic-recreational uses, lack of participatory approach, lack of public relations studies. In addition, the inability to identify protected areas is an important problem. Considering the current situation and management of protected areas, it is seen that there are significant problems in achieving an effective protected area management.

However, it is experiencing a change that cannot be ignored in the management and planning approaches of protected areas. As a result of scientific studies carried out in order to reveal the importance of protected areas, very important results are revealed. In the light of these data, it allows the use of concrete data in the planning of protected areas, which are very rich in natural resources, in visitor and especially in waste management. In addition, revealing the importance of protected areas within the forest area is clearly beneficial for planning these areas and producing policies. In addition, the preparation of participatory management plans and the adoption of local management will be tools that define sustainable development and ensure rational use.

Although the infrastructure services of protected areas are sufficient today, improvement and development investments should be programmed together with modeling studies for these services considering the coming years. In addition, in parallel with the developments in the literature, in future studies, color, shade and brightness analyzes should be made with GIS software using satellite images for the landscape beauty of protected areas. Thus, revealing more quantitative, objective and useful variables will contribute to the development of research on protected areas, especially in forests.

There is a lot of work on the planning and management of protected areas, especially in Europe. However, the planning and management of protected areas in Turkey has not been studied much. However, it is obvious that it makes a very high contribution to tourism mobility in the regions where protected areas are located. The deficiency in the economic contribution of the protected areas, which have a very rich natural resource in the forest areas, to tourism should be eliminated immediately. In addition, the future planning of protected areas, visitor and especially the use of concrete data in waste management is required. In this context, universities and research institutions in Turkey should increase the number of studies on green areas (such as forests), especially on the landscape value of protected areas.

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CHAPTER 6

IN VITRO APPROACHES FOR BIOACTIVE COMPOUNDS IN PLANTS

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1. Introduction

Plants, also known as 'green factories' have been utilized as a dependable and sustainable source of nourishment, flavoring, agrochemicals, and therapeutic substances since ancient times. The majority of the world's population gives plants and plant-derived constituents prominence for their wellness and youthfulness (Winter et al., 2012; Yuan et al., 2016; Chandran et al., 2020).

Plants produce a wide range of bioactive compounds, which are primarily classed as primary (essential) and secondary (non-vital) metabolites. Primary metabolites are necessary for fundamental functions like as photosynthetic activity, respiration, growth, and development.

Other phytochemicals are plant secondary metabolites, also known as 'specialized metabolites' which are tiny molecules with a variety of chemical structures and biological activity. The distribution of certain kinds of plant 'specialized metabolites' is generally confined to taxonomically related species since these substances are so diverse. Secondary metabolites in plants are an attractive source of phytochemicals with a wide range of chemical properties. These specialized metabolites are not as essential as primer metabolites for maintaining their life; however, they mediate between plant and environment interactions and might play a critical role in their survival and reproduction.

The primary activities of bioactive compounds are as follows:

✓ Competitive instruments against other living organisms including animals, plants, insects, as well as microbes

✓ Metal carrying agents

✓ Symbiotic connection agents

✓ Reproductive agents

✓ Differentiation effectors

✓ Organism-to-organism connection agents (Thirumurugan et al., 2018).

Green factories have always made a great contribution to both classic and modern medicines. They have many health-promoting and health-protecting characteristics as they are rich in these specialized metabolites. Also, as plants are phytochemicals-rich and have wonderfully competency to treat ailments and may be employed in a variety of industries, including medicines, cosmetics, agrochemicals, nutraceuticals, and so on (Nasri et al., 2014; Chandran et al., 2020) (Figure 1).

Due to their affordability, availability, eco-friendliness, and projected efficacy equivalent to high-cost synthetic pharmacological agents, bioactive compounds receiving greater attention among the rising population. The growing demand for such items has necessitated the development of ways to increase output without affecting their natural population (Atanasov et al., 2015; Chandran et al., 2020).

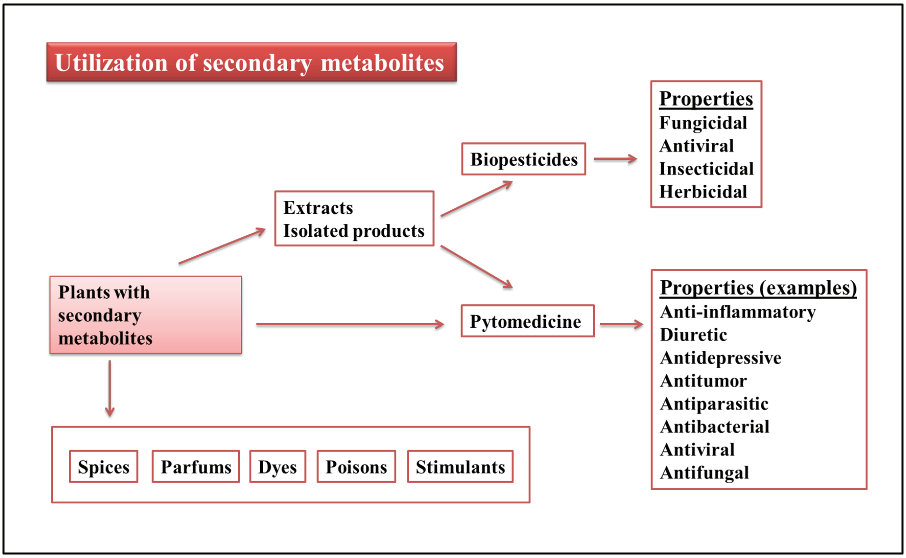

Figure 1. *Utilization of bioactive compounds in biotechnology (Adapted from Wink, 2010)*

Healthy and herbal items are becoming more popular in the growing market as consumers get more conscious of their lifestyle choices. Recently the attention to medicinal plants boosted due to their health benefits with regards to reliability and affordability when compared to synthetic medicines. (Ekor, 2014; Thomford et al., 2018; Anand et al., 2019; Chandran et al., 2020). The market of herbal medicine has recently emerged as one of the world's fastest-growing industries. It is expected the global market of medicinal plants and their valuable bioactive compounds to reach from 60 billion dollars (2000) to 5 trillion dollars (2050). Plant metabolites can be separated from spontaneously grown plants, however due to environmental and geographical limits, commercial production is restricted (Yue et al., 2016; Chandran et al., 2020). Traditional techniques take a long time since the plant must grow and mature for the required metabolite to be produced. Using plant tissue culture methods as an *in vitro* approach for the efficient manufacture of secondary metabolites in a short period of time for commercial use is an alternative option to overcome such a predicament (Verpoorte et al., 2002; Kolewe et al., 2008; Chandran et al., 2020). It allows for large-scale plant multiplication in controlled environments with no seasonal restrictions

(Murthy et al., 2014; Ochoa-Villarreal et al., 2016; Chandran et al., 2020).

It is due to their enormous diversity that specialized metabolites have piqued people's curiosity. They appear to be an endless supply of unique chemical structures with a wide range of pharmacological effects. More than 200,000 metabolites of this kind have been identified from higher plants. As stated before some secondary metabolites are only found in particular related plant species, and their quantities differ with plant ontogeny and tissue type in populations and individual plants. These secondary metabolites differentiations may be since genetic variability, but their concentrations are influenced by climatical and environmental conditions, abiotic and biotic factors. Specialized metabolites are divided into three categories based on their biosynthetic sources, chemical structures, and functions: terpenoids, phenolics, and nitrogen-containing compounds.

Together with the rising interest in plant-based compounds, direct secondary metabolite extraction at a wider scale has turned challenging since instability in environmental factors. Furthermore, secondary metabolite production is frequently confined to a single tissue and occurs at a certain stage of development. Extraction of important secondary metabolites from rare or almost extinct higher plants is similarly problematic. There may be numerous drawbacks in amounts of metabolites, such as low productivity, waving in their concentrations due to environmental, seasonal, and geographical conditions, when the secondary metabolites production takes place in open field or greenhouse cultivation of plants. Thence, alternate resources for the synthesis of specialized metabolites are in great demand. Using *in vitro* techniques, biotechnological synthesis of important specialized metabolites from such plants is a striking option (Ramachandra Rao & Ravishankar, 2002) (Figure 2).

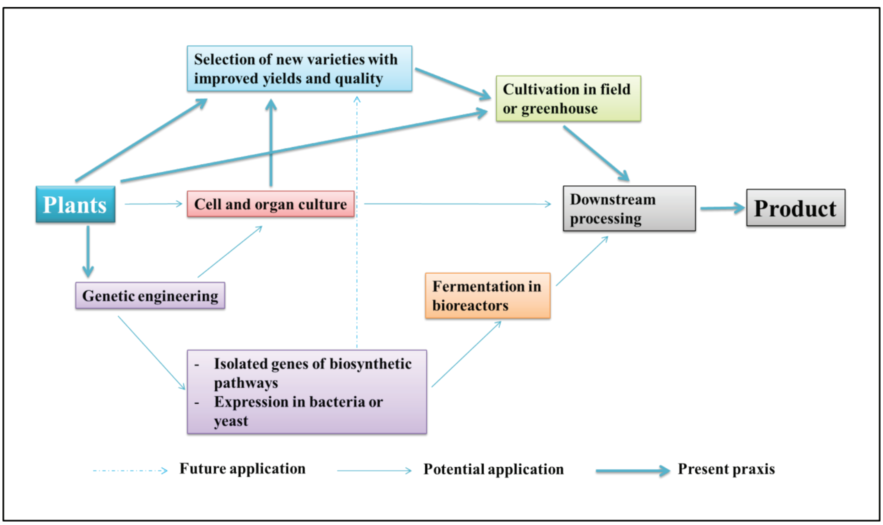

Figure 2. *Strategies for the production of bioactive compounds (Originally taken from Wink, 2010)*

2. *In vitro* systems and plant bioactive compound(s) production: plant cell and organ cultures

Plant cells and organ cultures have been improved as a bioactive compound synthesis alternative. The potential benefits of plant tissue culture for bioactive metabolites include; the desired production at desired times, standard quality product in a brief duration, independent development during the year regardless of environmental changes, preservation of natural resources of wild species on the verge of extinction, and the production of phytochemicals. Plant tissue culture methods can also be used to extract new plant chemicals that are not present in wild populations of species (Ramachandra Rao & Ravishankar, 2002; Akın, 2020). On the other hand, callus and cell suspension cultures may generate bioactive components, allowing them to manipulate bioactive compound production pathways (Chandran et al., 2020). Bioactive compounds have been purportedly produced using organized structures such as roots and shoots, calli, cell suspension, and so on. For obtaining bioactive components, callus production has been demonstrated to be more dependable than gathering plant materials from nature (Efferth, 2019; Chandran et al., 2020). *In vitro* callus induction, cell suspension cultures, and organ cultures can all be used to manufacture plant secondary metabolites. Plant cells and organ cultures have been created as a secondary metabolite manufacturing option. It is possible to ensure the long-term viability of essential medicinal and aromatic plants by lowering reliance on wild populations by manufacturing these substances using *in vitro* culture procedures (McDonald et al., 1995; Ramachandra Rao & Ravishankar, 2002; Nalawade & Tsay, 2004; Filová, 2014; Cardoso et al., 2019; Akın, 2020) (Figure 3).

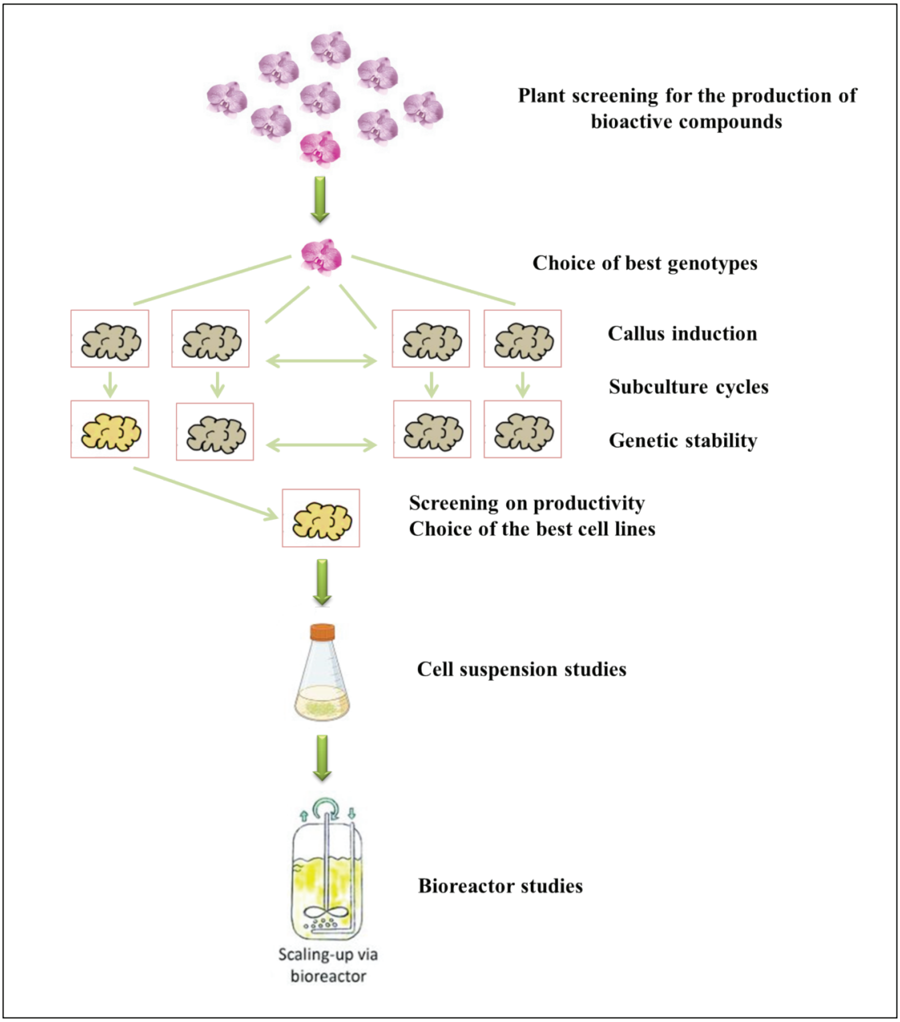

Figure 3. *Guidelines for the production of bioactive compounds via plant cell culture*

(Adapted from Bourgaud et al., 2001)

For compound synthesis, differentiated organ cultures such as shoots or roots have also been documented, and can reflect a compound pattern similar to natural plants (Karuppusamy, 2009). The most promising alternative approach for developing phytochemicals produced in plant roots is plant hairy root cultures (Pence, 2011). *Agrobacterium rhizogenes* intermediated transformation has been exploited to induce hairy roots in plants, allowing *in vitro* production of compounds generated in plant roots (Palazón et al., 1997; Chen et al., 2018). They have devised a useful biological method for investigating the production of a variety of bioactive chemicals, including nicotine and tropane alkaloids (Zhang et al., 2004; Yu et al., 2005), ginsenosides (Ha et al., 2016), anthraquinones (Perassolo et al., 2017) and artemisinin (Patra & Srivastava, 2016). Hairy roots are advantageous for compound formation because of their high output, consistency, and competence (Thiruvengadam et al., 2016; Thakore et al., 2017; Cardoso et al., 2019; Chandran et al., 2020). (Figure 4).

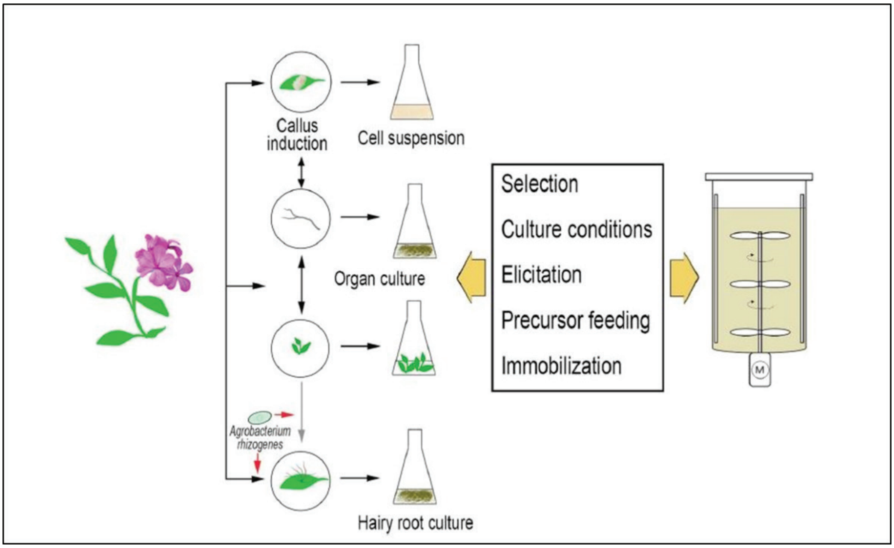

Figure 4. *Methods to produce bioactive compounds via plant in vitro culture (originally taken from Wawrosch & Zotchev, 2021)*

Unfortunately, many bioactive compounds are created in minute amounts through the producer organism, which might be slow-growing or difficult to cultivate in the laboratories. Metabolic engineering of living organisms has the capability to have been a scalable, selective, as well as cost-effective method of obtaining these high-value compounds with high yields and purity levels. Moreover, changes to the structure of bioactive compounds can frequently improve or alter the compound's biological action (O'Connor, 2015).

Applying different techniques for biomass increase and secondary chemical synthesis have been developed in recent years, including strain improvement, media and culture condition optimization, elicitation, nutrition and precursor feeding, permeabilization, immobilization, and biotransformation approaches (Figure 4).

The biomass increase and metabolite production by cultured plant cells and organs occur in a two-step process: (1) at first, cultured plant cells and organs participate in biomass growth, proliferation, and accumulation, as well as (2) metabolite synthesis from biomass (Table 1). Previously, the biomass and specialized metabolite production processes were carried out concurrently; however, by adopting the two-stage approach, both biomass growth and specialized metabolite accumulation may be improved (Murthy et al., 2014).

Table 1. *Approaches for increasing bioactive compounds synthesis in plant cell and tissue cultures*

Step 1	Step 2
✓ Obtaining, selecting and screening of efficient plant cell lines for high-growth	✓ Utilization of elicitors to boost productivity in a brief period
✓ Culture media optimization	✓ Nutrient feeding
o Effects of nutrition media and salt concentration	✓ Precursor feeding
o Carbohydrate supply and concentration	✓ Metabolites permeation for ease downstream processing
o Plant growth regulator types, levels and combinations	✓ Cells' immobilization to enhance extracellular bioactive compound production and promote biotransformation
o Effect of nitrogen source	✓ Plant cell and tissue cultures at a larger scale in appropriate bioreactors
o Effect of phosphate level	
✓ Effect of inoculum density	
✓ Optimization of cultural conditions	
o Effect of temperature	
o Effect of light intensity and quality	
o Effect of medium pH	
o Effect of agitation and aeration	

Adapted from Ramachandra Rao & Ravishankar, 2002; Murthy et al., 2014

Obtaining, selecting and screening of efficient plant cell lines for high-growth

Plant cell and tissue cultures are a heterogeneous group in which individual plant cells have diverse physiological features. Previously, selecting and screening of plant cell and tissue cultures were used to synthesize many products in large quantities. Cell cloning techniques offer a potential approach of choosing cell lines that produce higher quantities of output. To acquire high-producing cell lines, strain enhancement begins with the selection of a parent plant with increased levels of the required products for callus induction. The heterogeneous group is then screened for variant cell clones bearing the greatest quantities of the target product. Ogino et al., 1978 to create highly productive cell lines, variability in biochemical activity within a population of cells have used. It can be easily obtained if the concerning product is a pigment. Various strategies like mutation have also been utilized to acquire overproducing cell lines. Also, selective agents have been used as an alternative to traditional methods for selecting high-yielding cell lines (Ramachandra Rao & Ravishankar, 2002).

Culture media optimization

Environmental influences are known to have a significant impact on plant secondary metabolism. In plant cell and tissue cultures, nutritional components in the culture media, such as carbon, phosphate, nitrogen sources, and other macro elements, as well as plant growth regulators, such as auxins and cytokinins, impact both growth and metabolite synthesis. The concentration of plant growth regulators is frequently a major consideration in secondary metabolite accumulation (DiCosmo & Towers, 1984; Ramachandra Rao & Ravishankar, 2002). In cultivated plant cells, the depending on the concentration of auxin or cytokinin, as well as the auxin/cytokinin balance, has a significant impact on both growth and metabolite synthesis (Mantell & Smith, 1984; Ramachandra Rao & Ravishankar, 2002).

Effect of inoculum density

Regarding plant cell callus and suspension cultures, inoculum density is a critical aspect that influences growth, biomass yield, and bioactive compounds synthesis. Plant cell and/or organ development will often fail if the inoculum size is at below the threshold. To date, numerous researches have been conducted on the impact of inoculum density on biomass and metabolite formation in cultured cells. When cells are relocated to a different media, inoculum density is also considered to induce the stimulation of enzymes involved in general phenylpropanoid metabolism. These terms are referred to as "transfer effect" or "dilution effect".

Optimization of cultural conditions

Environmental (physical) elements can directly or indirectly impact *in vitro* bioactive component formation, in addition to chemical factors. Impact of culture environmental requirements such as illumunation, temperature, medium pH, agitation, and aeration are crucial factors on culture developments, bioactive compounds accumulation as well as large-scale production in several kinds of cultures. Of course, depending on the cultured plant, the type of culture, and even the age of the culture, each of these things has an impact.

Utilization of elicitors to boost productivity in a brief period

Secondary metabolites are produced by plants naturally as a defensive strategy against external factors. Elicitors are impulses that cause secondary metabolites to accumulate (Mulabagal & Tsay, 2004). An elicitor is a chemical that, when applied to a live cell system in tiny quantities, begins or enhances the formation of specific molecules (Namdeo, 2007). Elicitation is the induction or enhancement of bioactive metabolites by adding trace quantities of elicitors which is one of the most effective ways of increasing the efficiency of valuable metabolic compounds (Mulabagal & Tsay, 2004; Namdeo, 2007). Elicitors stimulate a variety of defensive responses by interacting with receptors in the plasma membrane of numerous plant cells. The potential of such elicitors to multitask is both unique and multifaceted. According to previous research, it has the ability to regulate a vast amount of biochemical control points, as well as activate the production of critical genes and transcription factors. Thence, it is now widely accepted that elicitors have the potential to affect a diverse range of cellular functions at the biochemical and molecular levels (Giri & Zaheer, 2016).

Although elicitors are characterized in a variety of ways, they are categorized as abiotic ('hormonal', 'physical' such as salinity, drought, thermal stress, UV radiation, osmotic, and water stress etc., and 'chemical' such as heavy metals, mineral salts and gaseous toxins) and biotic elicitors (polysaccharide, yeast extract, fungal and bacterial etc.) when classed according to their nature, and exogenous and endogenous elicitors when classified according to their origin (Namdeo, 2007; Akın, 2020). While elicitors affect the accumulation of the target bioactive component, they are affected by different parameters. Factors such as the concentration of the elicitor, the exposure time to the, the age of the culture to which the elicitor will be applied, and the composition of the medium may create differences in the desired secondary metabolite amount (Namdeo, 2007).

Precursor feeding

Precursor feeding has long been a well-known and widely used method for increasing bioactive compound synthesis in plant cells. Exogenous application of biosynthetic precursors to the culture and growth media may also boost bioactive product production (Mulabagal & Tsay, 2004). The theory is that any chemical that is an intermediary in or near the start of a secondary metabolite biosynthetic pathway has a fair possibility of improving the yield of the ultimate product. In many circumstances, providing precursor or intermediate substances to stimulate or boost the synthesis of bioactive phytochemicals has proved successful (Ramachandra Rao & Ravishankar, 2002).

Nutrient feeding

After adjusting the chemical and physical conditions for large-scale cell/organ growth, the media/nutrient feeding approach may be one of the numerous ways used to optimize bioactive compound production (Zhong, 2001; Jeong et al., 2008; Murthy et al., 2014). Both dry biomass and bioactive compound content have increased whenever the cultures were supplemented with the new medium. Various cell and tissue culture techniques, such as adventitious root cultures, cell cultures, and cell suspension cultures in numerous plant species, have shown a comparable, favorable impact of the medium exchange approach (Wang et al., 2001; Srinivasan & Ryu, 1993; Wu et al., 2007; Murthy et al., 2014).

Metabolites permeation for ease downstream processing

Phytochemicals produced by plant cell cultures are often kept in vacuoles; hence, extracting the compounds into the growth media in a way that makes the purification method easier is highly preferred. Removing bioactive components from the cell's vacuoles would also reduce product inhibition and boost productivity. The enzyme activity related to primary metabolism can also be used to evaluate cell permeability. Efforts have also been attempted to transiently permeabilize plant cells while maintaining cell viability and allowing for enhanced mass flow of substrate and phytochemicals to and from the cell for a brief period of time (Brodelius & Nilsson, 1983; Parr et al., 1987; Brodelius, 1988, Ramachandra Rao & Ravishankar, 2002). Permeabilizing agents include organic solvents like isopropanol and dimethylsulfoxide (DMSO), as well as polysaccharides like chitosan (Van Uden et al., 1990; Beaumont & Knorr, 1987; Knorr & Teutonico, 1986; Ramachandra Rao & Ravishankar, 2002). Ultrasonication, electroporation, and ionophoretic release are other permeabilization procedures wherein the cells are exposed to low power levels in a specifically built device (Brodelius et al., 1988; Ramachandra Rao & Ravishankar, 2002). Furthermore, the recovery of bioactive compounds has already been reported by employing strong electric field impulses and ultrahigh-pressure (Dornenburg & Knorr, 1993; Ramachandra Rao & Ravishankar, 2002).

Cells' immobilization to enhance extracellular bioactive compound production and promote biotransformation

The structure and differentiation of plant cells are frequently linked to improvements in bioactive compound synthesis in cell cultures. Immobilization technology, which already has long been utilized for microorganisms and enzymes, was inspired by the notion of organization and differentiation. Immobilization is a process that keeps a catalytically active enzyme as well as cells bound to a stable support and prevents them from entering the liquid phase (Yeoman et al., 1990; Yeoman, 1987; Fowler, 1986; Lindsey & Yeoman, 1985; Ramachandra Rao & Ravishankar, 2002). Individual and multiphase biotransformations of precursors to ambitioned products, along with de novo production of bioactive compounds, have all been accomplished using immobilized plant cells (Ramachandra Rao & Ravishankar, 2002). The most extensively used procedure is encasing cells in a gel or a mixture of gels that are enabled to polymerization reaction around them (Novais, 1988). The most common matrix is calcium alginate, while other gels that agar, agarose, gelatin, carrageenan, and polyacrylamide have also been employed (Nilsson et al., 1983; Ramachandra Rao & Ravishankar, 2002).

Biotransformation is described as a process in which live cultures, imprisoned enzymes, or permeabilized cells modify the functional groups of organic substances regioselectively or stereospecific to produce a chemically distinct result (Giri et al., 2001; Banerjee et al., 2012; Murthy et al., 2014). Throughout the biotransformation process, biological catalysts in the form of enzymes and entire cells can produce high-value food metabolites, fine chemicals, and medications (Berger, 1995; Krings & Berger, 1998; Meyer et al., 1997; Scragg, 1997; Ramachandra Rao & Ravishankar, 2002). It can be utilized from the biotransformation process to make food additives or medicines from cell suspension cultures, immobilized cells, enzyme preparations, and hairy root cultures.

Plant cell and tissue cultures at a larger scale in appropriate bioreactors

Plant cells suspended in liquid present a unique mix of environmental circumstances that must be adjusted in bioreactor procedures on a large scale. The volatility of productive cell lines, the difficulty of cell development, and a lack of understanding about the metabolic route are all well-known limitations of cell suspension cultures (Fulzele, 2005; Karuppusamy, 2009). For plant cell and tissue cultures, a number of bioreactor models have been developed and employed. In the development of plant cells, stirred tank reactors, bubble column reactors, airlift reactors, and flood reactors are basically modified extension microbiological culture techniques (Murthy et al., 2014).

3. Conclusions & Prospects

In vitro culture techniques are frequently utilized for large-scale generation of secondary phytochemicals in challenging or medicinally important plant species in a short time when compared to traditional methods. To accommodate the rising demand for all of these natural metabolites, numerous efforts have been used to develop plants with appealing metabolite-producing characteristics. Recent advancements in plant cell and tissue culture techniques and bioprocessing have already shown encouraging outcomes in terms of increasing biomass growth and performance. Several industries such as pharmaceuticals, cosmetics, agrochemicals, and nutraceuticals interested in exploiting natural compounds with key bioactive qualities might turn to plants for help. *In vitro* culture techniques, on the other hand, are crucial for protecting biodiversity and reproducing threatened extinction plant species. Over-exploitation of these valuable resources, besides, has the potential to reduce biodiversity and potentially lead to the extinction of significant herbals that accumulate essential compounds. *In vitro* culture techniques have long been regarded as a cost-effective method of producing phytochemicals. Utilizing the innovative *in vitro* approaches is still a prevailing strategy to become plant cells and organs into miniature bio-factories in the interest of such industrial production of bioactive compounds. The use of plant cell and organ culture techniques and a scale-up approach can result in a significant boost in bioactive compounds' productivity. The effective industrialization of plant cell bioprocesses will be aided by a thorough knowledge and study of these characteristics. In recent years, we have seen remarkable advancements in plant cell and tissue culture techniques. We are convinced that these breakthroughs will help to maintain biodiversity while also influencing the creation of pharmaceuticals, agrochemicals, and nutraceuticals utilizing plants. All these advancements are founded on the recognition of plants as irreplaceable natural resources in addition to contributing to satisfying cultural, financial, and marketing demands.

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CHAPTER 7

USE OF WASTEWATERS AND SALINE WATERS IN AGRICULTURE

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1. Introduction

Agriculture is the sector that uses the most freshwater in the world. About 2/3 of the freshwater is used in agriculture. The decrease of freshwater in semi-arid and arid areas of the world has reached a critical stage. The FAO estimates that the world's water need for irrigation will double by 2050 due to the increasing rural and urban population, economic growth of countries, and developments in lifestyles (Anonymous, 2022). In Turkey, 74% of freshwater is used for agriculture, 11% for industry, and 16% for domestic purposes. In agricultural irrigation, the use rate of surface water is 73%, groundwater use is 17%, and the use of drainage and wastewater is 10% (DSİ, 2021).

It is reported that around 20 million hectares of the cultivable area in the world are irrigated via wastewater. Unreported uses of wastewater in agriculture are estimated to be relatively high. Unclean water is used for agricultural purposes almost all over the world, especially in urban areas with scarce water. While efforts are still needed to advance national legislation, safe use guidelines, and practices, international societies have acknowledged that the safe use of wastewater in agriculture is a critical water resources issue that must be addressed. The safe use of wastewater and its valuable source is a fundamental point. The wastewater collection and treatment problem has been solved from a technological point of view. But, many countries do not have wastewater treatment technology (UNW-DPC, 2013).

Using water of poor quality will increase the water available for crops. Reusing agricultural drainage water, using wastewater, or using contaminated groundwater are all ways to accomplish this. The current water resources can be significantly increased by including these water resources in regional water usage plans. However, there are significant risks associated with using these waters, even though minimal usage of quality water can improve the supply available for agricultural output. Human activities, agriculture, and industry have polluted marginal quality water resources. Pollutants in these sources threaten human health, soils, and plants. Planners should be aware of the dangers when integrating these resources into agricultural water use policy. Different strategies should be tried for maximum benefit. Intelligent and comprehensive plans will benefit the protection of sustainable agriculture with these waters. (Abbott and Hasnip, 1997).

The decreased freshwater resources have become a fundamental problem in arid, semi-arid regions. Therefore, agricultural drainage water treated wastewater and shallow saline groundwater can significantly increase existing water resources. However, using these waters in irrigated areas requires salinity control through leaching and drainage of excess salt. However, the leaching of salts, micro soil elements, and agricultural chemicals may reduce drainage water quality. Deep percolation can also pollute groundwater. For these reasons, using saline waters requires comprehensive analysis (Tanwar, 2003).

There has been a significant increase in wastewater re-use in the world recently. The use of wastewater increased by 10 %to 29 %in Europe, China, and America and 41 % in Australia in a year (Zhang and Shen, 2017). Treated wastewater, which is used in significant amounts worldwide, especially in countries with water shortages, is generally used in small-scale agricultural activities in Turkey (Kukul ve ark., 2008; Yalılı Kılıç, 2020). Since a clean water supply is provided mainly in agricultural production in Turkey, this issue has not been addressed in all its aspects. However, in the coming years, wastewater use is predicted to increase, especially in arid regions of Turkey (Üstün and Solmaz, 2008).

2. Marginal Quality Waters

Agriculture can use less quality water than domestic and industrial purposes. The most common sources of marginal quality water are municipal and industrial wastewater, agricultural drainage water, and shallow groundwater (Quadir et al., 2007).

Salt and toxic ions are the primary pollutants in agricultural drainage and ground waters, while trace elements, organics, nutrients, and pathogens are the primary pollutants in the wastewaters. Marginal quality water resources can significantly impact plant, soil, and human health. With careful planning and comprehensive management, these water resources can be used for agricultural purposes. Salts from these pollutants threaten soil and plants; pathogens threaten crops and human health; trace elements, plant nutrients, and organic components threaten human health (Chapman, 1992). The following criteria must be established to use water of marginal quality for irrigation. (Table 1).

Table 1. *Conditions for the Use of Marginal Quality Water for Irrigation*

Parameter	Units
Total salinity	is analyzed in dS m^{-1}
Sodium	are analyzed in meq l^{-1}
Boron	
Chloride	
Calcium	
Magnesium	
pH	-
Trace element concentrations	are analyzed in mg l^{-1}
Nutrient levels	
Fecal coliforms	are analyzed in no. per 100 ml
Organic pollutants	are analyzed in µg/l
Intestinal nematodes	are analyzed in eggs per litre

The primary pollutants in marginal quality water used in agricultural irrigation are salts and toxic ions. Table 2. provides the FAO's guidelines for interpreting water quality for irrigation purposes.

Table 3 displays acceptable SAR and RSC limitations for agricultural water consumption.

Table 2. *FAO standards for irrigation water quality (Ayers and Westcot, 1985)*

Risk for irrigation problem			Effects	None	Mild to moderate	Intense
				Crop		
Salinity	EC_W	(dS m^{-1})		< 0.7	0.7 - 3.0	> 3.0
				Soil		
Infiltration	SAR = 0-3	and EC_W =		> 0.7	0.7 - 0.2	< 0.2
	3-6			> 1.2	1.2 - 0.3	< 0.3
	6-12			> 1.9	1.9 - 0.5	< 0.5
	12-20			> 2.9	2.9 - 1.3	< 1.3
	20-40			> 5.0	5.0 - 2.9	< 2.9
				Crop		
Specific ion toxicity	Sodium	SAR		< 3.0	3.0 - 9.0	> 9.0
	Chloride	meq/l		< 4.0	4.0 - 10.0	> 10.0
	Boron	mg/l		< 0.7	0.7 - 3.0	> 3.0
pH	Normal range =			6.5 - 8.4		

Note SAR: sodium adsorption ratio; EC_W: the electrical conductivity of water.

Tablo 3. *SAR and RSC thresholds that are appropriate for use in agriculture (Singh et al., 1996)*

Soil texture	Upper limits	
(% clay)	SAR (mmole/$l^{0.5}$)	RSC (meq/l)
Fine (>30%)	10	2.5 to 3.5
Generally good (20 to 30%)	10	3.5 to 5.0
Generally coarse (10 to 20%)	15	5.0 to 7.5
Coarse (<10%)	20	7.5 to 10
Explanation: *Saline Water means RSC < 2.5 meq/l;* *Sodic Water means RSC > 2.5 meq/l and ECw < 4.0 dS m^{-1}*		

Classification of water quality is a complex issue. A standard classification is not advised to determine if water is suitable for irrigation of agricultural land. Table 4 lists the criteria governing irrigation water quality and typical intervals.

Table 4. *Laboratory findings to assess the quality of typical irrigation water (FAO, 1985; Tanwar, 2003)*

Water element	Symbol	Used Unit[1]	The typical range for irrigation water
Salinity			
EC (Electrical Conductivity) TDS (Total Dissolved Solids)	ECw TDS	dS m^{-1} mg.1^{-1}	0 - 3 dS m^{-1} 0 – 2000 mg.1^{-1}
List of Nutrients[2]			
Nitrate-Nitrogen	NO_3-N	mg.1^{-1}	0/10 mg.1^{-1}
Phosphate-Phosphorus	PO_4-P	mg.1^{-1}	0/2 mg.1^{-1}
Ammonium-Nitrogen	NH_4-N	mg.1^{-1}	0/5 mg.1^{-1}
Potassium	K^+	mg.1^{-1}	0/2 mg.1^{-1}
Various			
Boron	B	mg.1^{-1}	0/2 mg.1^{-1}
State of acid-base	pH	1 / 14	6.0/8.5
Sodium Adsorption Ratio[3]	SAR	$(me/1)^{1,2}$	0/15
Anions - Cations			
Calcium	Ca++	mg.1-1	0/20 mg.1-1
Magnesium	Mg^{++}	mg.1^{-1}	0/5 mg.1^{-1}
Sodium	Na^+	mg.1^{-1}	0/40 mg.1^{-1}
Carbonate	CO_3^{--}	mg.1^{-1}	0/1 mg.1^{-1}
Bicarbonate	HCO_3^{---}	mg.1^{-1}	0/10 mg.1^{-1}
Chloride	$C1^{--}$	mg.1^{-1}	0/30 mg.1^{-1}
Sulphate	SO_4^{---}	mg.1^{-1}	0/20 mg.1^{-1}

The US salinity laboratory scheme is given in Table 5 to classify irrigation water quality in its modified form. This scheme is considered more appropriate for countries where most of the water is in a low salinity range (with some small changes).

Table 5. *Revised salinity laboratory water classification (US) (Tanwar, 2003)*

Class	Salinity		Evaluations
	m mhos/em	mg/l	
	> 6000	> 3500	Excessive (Not usable)
	4000/6000	2500/3500	Highly High (Only with very permeable soils and highly tolerant ones)
C_1	< 250	< 200	Low (Suitable for many crops)
C_2	250/ 750	200/500	Medium (May leaching needed for sensitive ones)
C_3	750/2250	500/1500	High (Tolerant ones and leaching)
C_4	2250/4000	1500/2500	High (Just useable with permeable soils and tolerant ones)

Table 6 lists the saline water classification that the FAO (1992) suggested.

Table 6. *Saline water classification based on salinity risk (FAO, 1992; Tanwar, 2003)*

Classes of water	EC_w (dS/m)	Salt concentration (mg/l)	Water Type
1	< 0.7	< 500	For drinking and irrigation
2	0.7 to 2	500 to1500	Irrigation water
3	2 to10	1500 to 7000	Firstly drainage water and groundwater
4	10 to 25	7000 to 15000	Medium drainage water and groundwater
5	25-45	15000 to 35000	Highly saline water
6	> 45	> 35000	Sea water
1: (NS) Non-saline 2: (SS) Slightly saline 3: (RS) Relatively saline 4: (HS) Highly saline 5: (VHS) Very high saline 6: Saline water = brine			

2.1. Wastewater Use

Urban areas are the largest source of wastewater contamination, and over 80% of wastewater worldwide is not collected or treated (WWAP, 2012). The increasing urban population affects wastewater production, treatment, and use in various ways. Large amounts of wastewater are both produced and consumed in urban areas. Greater urban water demands brought on by denser populations result in higher volumes of untreated or only partially treated effluent being released into the environment and water supplies. As a result, freshwater resources for agriculture and urban use are disrupted, and conventional irrigation water resources are contaminated. However, different types of wastewater, categories, and uses exist based on its composition, treatment, and intended or unintended utilization. Some information about the areas, agricultural areas, irrigation, population, and wastewater situation of Turkey is given in Table 7.

Table 7. *Some information about the areas, agricultural areas, irrigation, population, and wastewater situation of Turkey (FAO, 2017)*

Unit	Description	Data
Area	The total area of the country (1000 ha)	78 535
	Arable land (1000 ha)	19 723
	Total cultivated (1000 ha)	23 180
	The proportion of the country's total area that is used for farming (%)	29.52
	Cultivated land irrigated (%)	18.14
Population	Total (1000 inhabitant)	82 340
	Rural (1000 inhab.)	20 362
	Urban (1000 inhab.)	61 555
	Precipitation average over the long term (mm/year)	593

Table 7. *Continues*

Unit	Description	Data
	(NRI) - National Rainfall Index ($mm.yr^{-1}$)	614.5
	Total resources for renewable water (10^9 m^3·$year^{-1}$)	211.6
	Dependency ratio (%)	1.518
	Per capita total renewable water resources (m^3/inhab./yr)	2 570
Withdrawal (10^9 m^3·$year^{-1}$)	Agricultural water	51.73
	as a proportion of all water (%)	87.12
	Industrial water	3.076
	as a proportion of all water (%)	5.18
	Community water (Municipal water)	6.588
	as a proportion of withdrawal (%)	11.09
	Total water	59.38
	per capita (m^3/year per inhab.)	721.2
	Fresh surface water	44.91
	Fresh groundwater	16.18
	Total freshwater	61.09
	Irrigation water	43.95
Requirement (km^3/year or (10^9 m^3·$year^{-1}$)	Irrigation water	25.14
	Environmental Flow	76.97
Produced (km^3/year or (10^9 m^3·$year^{-1}$)	Municipal wastewater	5.28
	Desalinated water	0.0088
Direct use (km^3/year or (10^9 m^3·$year^{-1}$)	Treated municipal wastewater	0.0454
	Agricultural drainage water	-
	Municipal wastewater treated	0.0454
	Treated urban sewage water used for irrigation	0.0454
	Not using municipal wastewater treatment for irrigation	-
Municipal wastewater (m.w.) (km^3/year or (10^9 m^3·$year^{-1}$)	Collected	4.795
	Treated	4.236
	Not treated	0.814
	Treated m.w.discharged (secondary water)	1.591
	Not treated m.w. discharged (secondary water)	-
	The number of m.w. treatment facilities	604
	The capacity of the m.w. treatment facilities	5.289
Area equipped (1000 ha)	Utilizing directly treated m.w for irrigation	9.16
	For irrigation, using untreated m.w directly	9.16
	For irrigation: actually irrigated	5 280
	Drainage of an irrigation-ready area (1000 ha)	340.9
Area equipped (%)	Utilizing directly treated m.w for irrigation	0.1711
	For irrigation using untreated m.w. directly	0.1711
	Using agricultural drainage water directly for irrigation	-
	Desalinated water is used for irrigation.	-
	For irrigation salinized	28.38
	Irrigation potential (1000 hectares)	8500
	Area salinized by irrigation (1000 ha)	1519
Access to safe drinking water (JMP) (%)	Total population	100
	Rural population	100
	Urban population	100
	Population affected by the water-related disease (1000 inhab.)	-

Wastewater from various sources, including wastewater produced by diverse urban activities, is used for agricultural irrigation (from raw to dilution) (Raschid-Sally and Jayakody, 2008).

Usually, a combination of one or more of the above, urban wastewater includes:

- Blackwater (urine, sludge, etc.) and greywater (bath and kitchen wastewater) comprise domestic wastewater.

- Wastewater is caused by commercial institutions and organizations, including hospitals.

- Industrial wastewater.

- Rainwater and other urban currents.

To lessen the impact of harmful pollutants, treated wastewater is processed in a wastewater

treatment facility using physical, chemical, and biological processes.

Municipal wastewater that has been treated and is safe to use again is reclaimed water. Gray water is particularly suitable for reusing. It comes from households, can be treated, has no significant impurities, isn't connected to the sewage system, and can be used to water trees like olive trees and home gardens. Gray water is a crucial part of water conservation. It accounts for 50–80% of residential wastewater and has great promise as an economical and resource-protecting element of integrated water resources management in arid regions.

2.1.1. Benefits and Hazards of Wastewater Use

The well-managed wastewater can increase food safety and benefit society, the economy, and the environment. When alternative water resources are scarce in metropolitan regions, wastewater provides a low-cost resource that farmers may access year-round (UNW-DPC, 2013). By relieving demand on freshwater sources, wastewater in agricultural irrigation supplies water and nutrients to plants, supplying food to cities. Since treated wastewaters are rich in plant nutrients such as nitrogen and phosphorus, it reduces the need for fertilizers during agricultural use and increases the yield of the product received (Büyükkamacı, 2009; Demir et al., 2017, Adalı and Yalılı Kılıç, 2020). Wastewater contains plant nutrients that can increase crop growth and reduce the use of chemical fertilizers. Compared to irrigation with clean water, wastewater use can save up to 45% of fertilizer applied for wheat and up to 94% for alfalfa (Balkhair et al., 2013). Wastewater can supply soil and plants with large amounts of macronutrients (N, P, and K). Thus, production costs are reduced, but product losses can be 10-20%, depending on wastewater quality. However, the heavy metal and persistent organic pollutant content of wastewater limit the possibility of using treated wastewater in agricultural production due to its effects on the environment and human and animal health (Aydin et al., 2015; Adalı and Yalılı Kılıç, 2020). When wastewater is injected into the soil, it can build up in the soil, plants, and other living things, causing a hazard since removing harmful chemicals from wastewater happens at a specific rate during wastewater treatment. In the agricultural use of treated wastewater, attention should also be paid to pH, dissolved oxygen, and substance content. Table 8 lists the classifications of treated wastewater irrigation and quality characteristics by the Water Pollution Control Regulation (WPCR).

Table 8. *Classes and quality standards for treated wastewater irrigation by WPCR (SKKY, 1991)*

	Water for Irrigation Class				
Quality Standards	**1. Class (Perfect)**	**2. Class (Good)**	**3. Class (Usable)**	**4. Class (Usable with prudence**	**5. Class (Harmful/ Not suitable)**
EC25x10^{6}	0/250	250/750	750/2000	2000/3000	> 3000
(CSR) Changeable Sodium Ratio (% Na)	< 20	20/40	40/60	60/80	> 80
Sodium Adsorption Ratio (SAR)	< 10	10/18	18/26	> 26	
(RSC) The residue of Sodium Carbonate					
mg.1^{-1}	< 66	66/133	> 133		
meq/L	> 1.25	1.25/2.5	> 2.5		
Chloride (Cl^{-})					
mg.1^{-1}	0/142	142/249	249/426	426/710	> 710
meq/L	0/4	4/7	7/12	12/20	> 20
Sulfate ($SO_4^{'}$)	0/4	4/7	7/12	12/20	> 20
meq/L	0/4	4/7	7/12	12/20	> 20
mg.1^{-1}	0/192	192/336	336/575	575/960	> 960
(TSC) Concentration of Total Salt (mg.1^{-1})	0/175	175/525	525/1400	1400/2100	> 2100
Concentration of boron (mg.1^{-1})	0/0.5	0.5/1.12	1.12/2.0	> 2.0	-
Water for Irrigation class	C_1S_1	C_2S_1, C_1S_2, C_2S_2,	C_3S_1, C_3S_2, C_3S_3, C_1S_3, C_2S_3,	C_4S_3, C_4S_2, C_4S_1, C_1S_4, C_2S_4, C_3S_4, C_4S_4,	-
NO_3 or NH_4^{+} (mg.1^{-1})	0/5	5/10	10/30	30/50	> 50
Fecal Coliform 1/100 ml	0/2	2/20	20/100	100/1000	> 1000

BOİ$_5$ (mg.1^{-1})	0/25	25/50	50/100	100/200	> 200
Suspended Solids (SS) (mg.1^{-1})	20	30	45	60	> 100
pH	6.5/8.5	6.5/8.5	6.5/8.5	6.5/9	< 6 veya > 9
Temperature (°C)	30	30	35	40	> 40

The major alteration of the soil's physical, chemical, and biological characteristics due to wastewater may also alter the biogeochemical behavior of metals and other foods (mobility and bio-agglomeration). Therefore, due to wastewater application, the change in soil properties may significantly affect soil quality and product efficiency positively and negatively (Becerra-Castro et al., 2015).

Unwanted bacteria and chemical elements in wastewater can threaten human health and the environment. Also, worms, bacteria, viruses, and protozoa can be found in wastewater (Khalid et al., 2018). Vegetables and crops irrigated with wastewater contain potentially toxic elements (PTES) in high concentrations that may risk human health (Table 9).

It is advised to use wastewater treated in an amount that will meet the irrigation water quality requirements stated in the "Water Pollution Control Regulation Technical Procedures Communiqué" in areas where irrigation water is scarce and has economic value (SKKY, 2004). To minimize the effect the treated wastewater may have on the environment and public health in agricultural use, it should be purified from pathogens and microorganisms in its content by subjecting it to advanced treatment processes and biological treatment (Nas and Yılmaz, 2019; Adalı and Yalılı Kılıç, 2020).

Table 9. *The health danger criteria for vegetables cultivated using wastewater (Khalid et al., 2018)*

Metal	**Vegetable**	**Index of Health Risk**	**Daily Estimated Intake**	**Risk Quotient**	**Reference**
Cu	Cupressus sempervirens	0.1233	0.00493	0.001351	Farahat and Linderholm, 2015
	Raphanus sativus	0.0157	0.00063	0.000172	Qureshi et al., 2016
	Raphanus sativus	0.1180	0.00472	0.001294	Khan et al., 2008
	Lactuca sativa	0.1056	0.00422	0.001157	Akoto et al., 2015
	Xanthium strumarium	0.0104	0.00042	0.000114	Nazir, 2015
	Vicia faba	0.0005	0.00002	0.000006	Khan, 2013
	Citrus x sinensis	0.0571	0.00228	0.000625	Christou, 2014

Pb	Triticum	0.4152	0.00145	0.000398	Meng, 2016
	Raphanus sativus	0.3897	0.00136	0.000374	Khan et al., 2008
	Triticum	3.9315	0.01380	0.003770	Qishlaqi, 2008
	Convolvulus arvensis	0.2148	0.00075	0.000206	Abbasi, 2013
	Triticum	0.3447	0.00121	0.000331	Ma, 2015
	Oryza sativa	0.0555	0.00019	0.000053	Chung, 2011
	Cupressus sempervirens	0.4796	0.00168	0.000460	Farahat and Linderholm, 2015
Zn	Raphanus sativus	0.0997	0.02990	0.008192	Khan et al., 2008
	Daucus carota	0.0044	0.00131	0.000359	Qureshi et al., 2015
	Amaranthus	0.1172	0.03510	0.009630	Abdu, 2011
	Beta vulgaris	0.0437	0.01310	0.003593	Rodda, 2011
	Hordeum vulgare	0.0563	0.01690	0.004628	Rusan, 2007
	Citrus x sinensis	0.0073	0.00218	0.000596	Christou, 2014
Ni	Cupressus sempervirens	0.1233	0.00247	0.000676	Farahat and Linderholm, 2015
	Raphanus sativus	0.2885	0.00577	0.001581	Khan et al., 2008
	Zea mays	0.0695	0.00139	0.000381	De Melo, 2007
	Abelmoschus esculentus	0.0367	0.00073	0.000201	Balkhair, 2016
	Vicia faba	0.0024	0.00005	0.000013	Khan, 2013
	Triticum	0.7132	0.01430	0.003908	Qishlaqi, 2008
Cd	Cupressus sempervirens	0.0315	0.00003	0.000009	Farahat and Linderholm, 2015
	Raphanus sativus	0.4879	0.00049	0.000134	Khan et al., 2008
	Vicia faba	0.0525	0.00005	0.000014	Khan et al., 2008
	Oryza sativa	0.5771	0.00058	0.000158	Hu, 2014
	Spinach oleracea	7.8690	0.00787	0.002156	Abedi-Koupai, 2015
	Lactuca sativa	0.1049	0.00011	0.000029	Abdu, 2011

2.2. Saline water use

Despite being a reliable source of water, groundwater is vulnerable to contamination. Seawater can impact coastal locations, while groundwater may penetrate farming areas with nutrients, salts, harmful ions, and organic compounds. Nitrate is the most active of nutrients in the soil and is a common groundwater pollutant. Agricultural drainage water has entered the drainage system naturally or artificially after passing through the soil profile. Salt and toxic ions are the primary pollutants likely to be picked up when water passes through the soil profile.

Saline waters in large quantities in the world constitute a potential resource for irrigation. These waters, classified as very saline for conventional agriculture in the past years, are now used in irrigation. Depending on the salinity level, salt water can be utilized for irrigation by combining it with fresh water; alternatively, it can be used for irrigation without dilution. Another application uses saline waters with fresh water alternately (Zwart and Bastiaanssen, 2004; Qadir and Oster, 2004; Ma et al.., 2008; Rhoades et al., 1992; Malash et al., 2005, İnce Kaya, 2015).

Irrigation with saline water differs from other types of irrigation in that it uses salt water. The correct irrigation technique must be intended, modern irrigation technologies must be used when necessary, and protective measures must be taken to preserve the soil's physical features. Tolerant plants that can thrive in these saline circumstances must also be chosen. High salt concentrations in irrigation water can cause soil salinity to reach levels that will damage many cultural plants. The salinity in the plant root area causes low osmotic potential, which makes water intake with roots difficult. This has similar effects to drought effects in plants. On the other hand, the plant takes salt ions such as sodium, chlorine, and boron in the soil, which can have toxic effects. (Taiz and Zeiger, 2008, İnce Kaya, 2015). When plants are watered with saline water, the salinity level in the root zone does not rise uniformly; instead, a salinity gradient occurs. The conductivity of irrigation water, the amount of leaching treatments, and the previous soil qualities all impact the gradient's direction and slope. Guidelines have been produced for several plants for germination, emergence, and later growth periods since the sensitivity of plants to salinity might vary according to growth periods (Table 10).

Therefore, the bulk of crop salt tolerance recommendations take the following shape:

Yr = 100 - b(ECe - a)

Yr = Relative crop yield (%)

a= Salinity threshold value (dS.m^{-1})

b= Slope expressed in % per dS.m^{-1}

Ece= Electrical conductivity of a saturated soil extract (dS.m^{-1})

Table 10. *Crops' emergence and growth to maturity: relative salt tolerance*

Crop	**Salinity levels necessary for a 50 % yield (ECe dS m^{-1})**	**Salinity level required for 50% emergence (ECe dS m^{-1})**
Barley	18	16 / 24
Cotton	17	15
Sugarbeet	15	6 / 12
Wheat	13	14 / 16
Maize	5.9	21 / 24
Onion	4.3	5.6 / 7.5
Rice	3.6	18
Bean	3.6	8

Tables 11, 12, and 13 include information on the relative salt and chloride tolerance of various crops and the salinity levels of irrigation water acceptable for specific crops.

Table 11. *Salt tolerant levels of some crops (Ayers and Westcot, 1985)*

Tolerant: Cotton, barley, asparagus, date palm, sugarbeet
Semi-tolerant: Wheat, sorghum, barley, squash, berseem
Sensitive: Potato, maize, bean, flax, paddy rice

Table 12. *Allowable irrigation water salinity levels (Rao et al., 1994)*

Texture (% clay)	Plant Tolerance	Upper limit of EC_W (dS m^{-1})*		
		550 to 750 mm	350 to 550 mm	<350 mm
Fine	A	1.5	1.0	1.0
(>30%)	B	3.0	2.0	1.5
	C	4.5	3.0	2.0
Relatively fine	A	2.5	2.0	1.5
(20 to 30%)	B	4.5	3.0	2.0
	C	8.0	6.0	4.0
Relatively coarse	A	3.0	2.5	2.0

Table 12. *Continues*

Texture (% clay)	Plant Tolerance	The upper limit of EC_W (dS m^{-1})*		
		550 to 750 mm	350 to 550 mm	<350 mm
Fine	A	1.5	1.0	1.0
(>30%)	B	3.0	2.0	1.5
	C	4.5	3.0	2.0
Relatively fine	A	2.5	2.0	1.5
(20 to 30%)	B	4.5	3.0	2.0
	C	8.0	6.0	4.0
Relatively coarse	A	3.0	2.5	2.0
(10 to 20%)	B	8.0	6.0	4.0
	C	10.0	8.0	6.0
Coarse	A	3.0	3.0	-
(<10%)	B	9.0	7.5	6.0
	C	12.5	10.0	8.0

A: Sensitive B: Semi-tolerant C: Tolerant
* According to total annual rainfall

Tablo 13. *Relative salt and chloride tolerance*

Plant	Limit value[1] ECe (dS m^{-1})	Surface slope[1] (% per /dS m^{-1})	Threshold chloride ion concentration[2], **	Percent decrease in yield[2, **]
Barley	8.0	5.0	80	0.5
Tomato	0.9	9	-	-
Carrot	1.0	14	10	1.4
Onion	1.2	16	10	1.6
Berseem	1.5	5.7	15	0.6

Broad Beans	1.6	9.6	15	1.0
Maize	1.7	12	15	1.2
Flax	1.7	12	15	1.2
Rice	3.0	12	-	-
Wheat	4.5	2.6	60	0.7
Sugarbeet	7.0	5.9	70	0.6
Cotton	7.7	5.2	75	0.5
[1](Tanji, 1990) [2] (Maas, 1990)				
*When yield loss commences (meq/l) in saturated soil extract ** at Cl-concentrations above the threshold				

Malash et al. (2005) reported that the salt distribution in the soil differed depending on the irrigation method. According to Shallavet (1994), combining salt water with a drip irrigation system has many benefits. In the drip irrigation technique, plant leaves are not damaged by salt. However, in sprinkler irrigation, plant leaves are exposed to salt. The drip irrigation method wets the plant's root zone and adds less salt to the soil than the surface and sprinkler watering methods. In addition, the drip irrigation system enables automation and advanced technologies, control of surface flow losses, and leaching applications more efficiently.

The utilization of saline fluids depends heavily on leaching applications. Letey et al. (2011) reported that a practical leaching application is necessary to keep the salt balance in the soil at a level that does not damage the plant and prevent soil deterioration and possible salinity problems. Moreover, if natural drainage is missing in the area, an artificial surface, subsurface drainage system, or an integrated drainage system may be employed.

3. CONCLUSION

Using wastewater and saline waters in agricultural irrigation will significantly contribute to eliminating the global water deficit. In many regions of the world, waste irrigation helped solve the problem of water scarcity. Environmental and health problems result from the watering of agriculture with untreated wastewater. Risks will be reduced by using the right treatment and irrigation technologies. It is also necessary to note that sufficient and more strict legal arrangements should be applied for wastewater.

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CHAPTER 8

PARASITE BIODIVERSITY OF MARINE, FRESHWATER AND AQUARIUM FISHES IN TURKEY

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1. INTRODUCTION

Turkey is a country bridging the two continents Europe and Asia and it is located at a latitude of 38.9637° N, and a longitude of 35.2433° E with about 780 000 km^2 surface area (https://www.dsi.gov.tr/Sayfa/Detay/754). Turkey also has the longest coastal line among the EU, the Black Sea, the Mediterranean, and Adriatic Sea countries with 8,333 km long and is bordered by four different seas: the Mediterranean Sea (1707 km), the Black Sea (1701 km), the Aegean Sea (3484 km), and the Marmara Sea 1441 km), which is connected to the Black Sea by the Bosphorus Strait and to the Aegean Sea by the Dardanelles Strait (Güven et al., 2007; Akengin et al., 2016).

There are 25 freshwater basins most of which are born within the borders of Turkey and then pour into the surrounding above-mentioned seas. The main rivers poring to these seas in Turkey are Kızılırmak (1151 km), Sakarya (824 km), Büyük Menderes (584 km), Seyhan (560 km), Yeşilırmak (519 km), Ceyhan (509 km), Gediz (275 km), and Küçük Menderes (129 km). On the other hand, there are some rivers which are born within the borders of Turkey and then poring to seas surrounding other countries and these are; Fırat (part of 1 263 km in Turkey), Dicle (part of 512 km in Turkey), Çoruh (part of 354 km in Turkey), Kura (part of 189 km in Turkey) and Aras (part of 548 km) rivers. Turkey also inhabits about 320 natural lakes some of which Van Lake (3713 km^2), Salt Lake (1300 km^2), Beyşehir Lake (656 km^2), Eğirdir Lake (482 km^2) are among the largest ones.

These large amounts of freshwater and marine ecosystems in Turkey inhabit a total of 401 and 561 fish species, respectively (Faroese & Pauly, 2022). Among these great varieties of fish diversity in both environments, some fish species have been regarded as valuable food sources for human consumption and cultured in either land-based culture facilities and/or net cages in lakes and seas. Rainbow trout *Oncorhynchus mykiss*, European seabass *Dicentrarchus labrax*, and gilthead sea bream *Sparus aurata* are among the most cultured fish species in Turkey and their annual production values in 2019 are 123573 tonnes, 137419 tonnes, and 99.730 tonnes, respectively (TÜİK, 2020). Several other fish species such as meagre *Argyrosomus regius*, bogue *Boops boops*, sharp snout sea bream *Diplodus puntazzo*, white seabream *Diplodus sargus*, common pandora *Pagellus erythrinus,* and salema *Sarpa salpa* are also cultured in marine environments as new alternative food sources.

In freshwater and aquarium environments, several other fish species such as Black Sea salmon *Salmo labrax* and guppy *Poecilia reticulata,* etc. are also cultured for either food sources or ornamental fisheries in Turkey. For small-sized aquariums, highly diverse fish species are also imported and this has a high economic value throughout Turkey. As mentioned above, very diverse fish species in marine, freshwater, and aquarium environments in Turkey have been accepted as valuable sources either both for wild fish capture or fish culture and, thus, scientific studies are mainly focused on their anatomy, physiology, biology, and improvements on their culture methods either for already cultured ones or alternative fish species.

The number of studies by researchers on the parasite fauna of fishes have gained momentum in the last decades and several checklists of parasites of all the taxa and their respective hosts in freshwater and marine environments have been published by Öktener (2003, 2004, 2014), Özer & Öztürk (2017), Özer (2019, 2020). Moreover, Özer (2021) has recently published a very comprehensive host-parasite as well as parasite-host checklist book based on all previous reports in Turkey and this chapter has been created based on the data presented in this recent book.

2. Total parasite diversity of fishes in Turkey

According to Özer (2021), the highest number of parasite species was 400 from marine fishes, followed by wild freshwater, cultured marine, cultured freshwater, and aquarium fishes (Figure 1). It must be noted that out of 624 nominal and genus levels identified species of parasites in total, some parasites were found to be able to infect marine, freshwater, and aquarium fishes. A total of 287 different nominal and genus level fish species from both wild and cultured marine and freshwater, as well as aquarium, were reported to be hosting at least one parasite species in Turkey (Figure 2). Wild marine fishes were the most parasite reported hosts and followed by wild freshwater and aquarium fishes (Figure 2). The numbers of parasite species belonging to higher taxa from wild and cultured marine and freshwater fishes as well as aquarium fishes are presented in Figure 3.

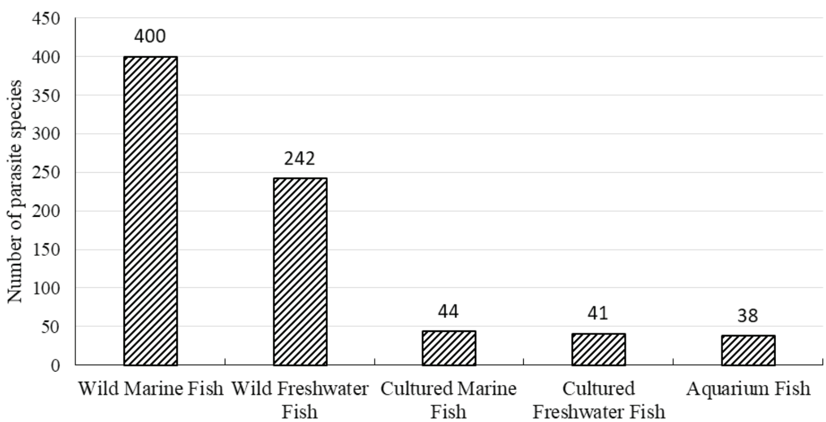

Figure 1. *The number of parasites species described from marine, freshwater, and aquarium fishes in Turkey.*

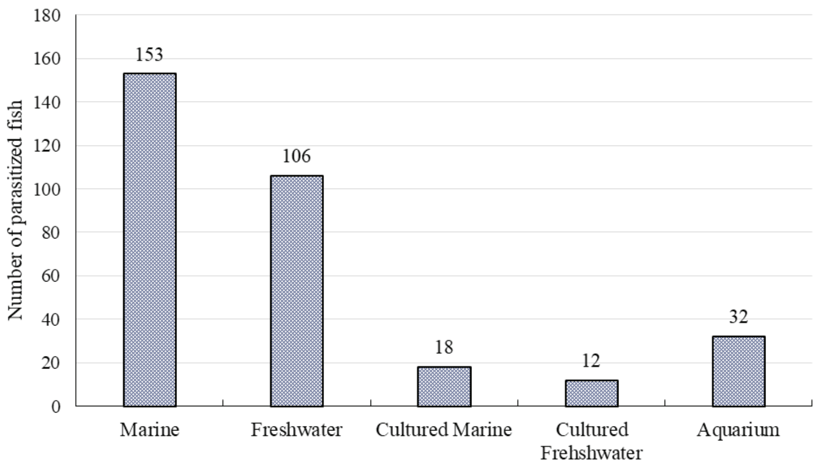

Figure 2. *The number of parasite-reported marine, freshwater, and aquarium fish species from different environments in Turkey.*

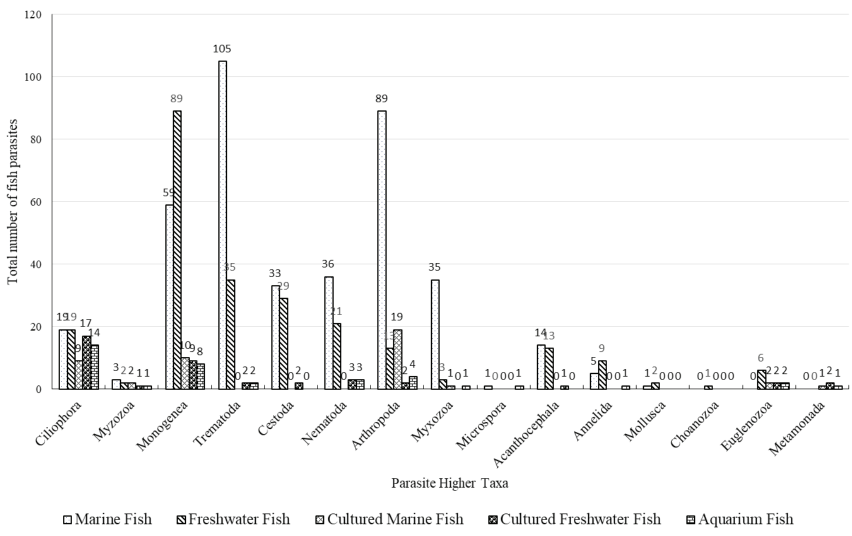

Figure 3. *The number of parasite species belonging to higher taxa infecting wild and cultured marine, freshwater, and aquarium fish species in Turkey.*

3. Parasite diversity of marine fishes

3.1. Wild marine fishes

A total of 153 wild marine fish species were reported to be the host of at least one parasite species and among them, a mugilid fish *Mugil cephalus* had the highest parasite diversity of 42 species, many other fishes belonging to very diverse families were also found to be infected with high numbers of parasites (Figure 4). It must be noted that the following highest parasite numbers were reported mostly from demersal fishes belonging to different families (see Figure 4 for details) and the lesser number of parasite species than 10 parasite species infecting marine host fishes in Turkey can be seen in the book published by Özer (2021).

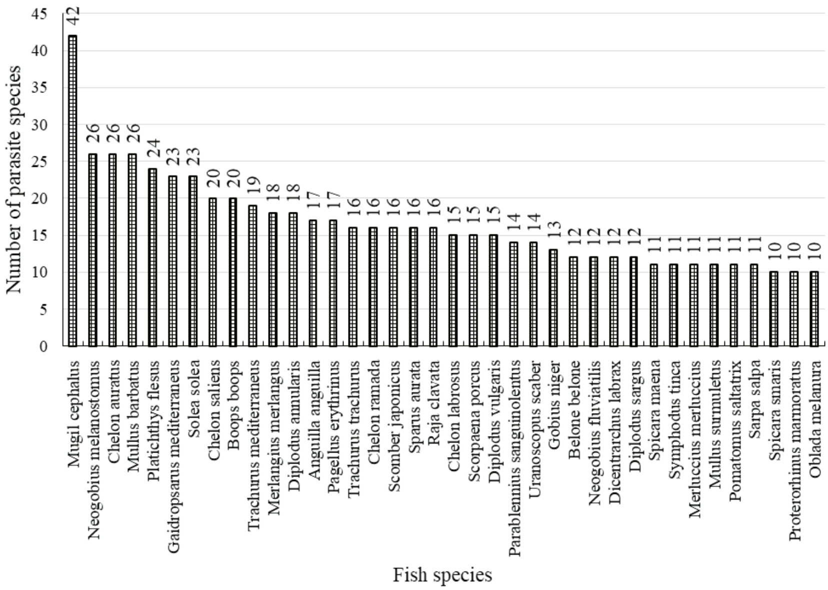

Figure 4. *The number of parasite species infecting the wild marine fishes in Turkey*

Wild marine fish subjected to parasitological investigation yielded a wide range of parasite species belonging to 12 higher taxa and the most specious is the trematodes with 105 species and followed by Arthropoda, Monogenea, Nematoda, Myxozoa, and Acanthocephala and others with very low numbers (Figure 5). Following the trematode parasites which have complex life cycles involving several other hosts, Arthropoda and Monogenean parasites which have direct life cycle strategies involving only fish host and water environment were the second and third parasite taxa to be reported from fish hosts (see Figure 5 for details).

Host specificity is a well-known issue for some of the fish parasites such as monogeneans and, on the other, some parasites were reported to infect at least one host species in their life cycle.

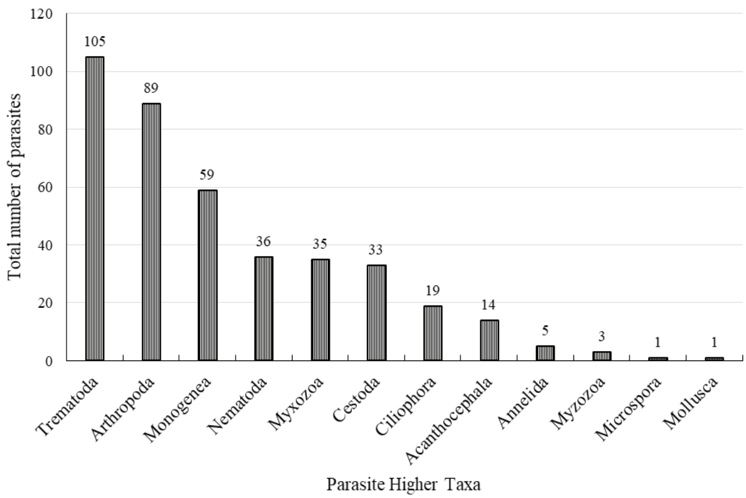

Figure 5. *The number of parasite species belonging to higher taxa infecting wild marine fishes in Turkey*

Figure 6 illustrates parasite species with broad host diversity and the most cosmopolitan two species, *Hysterothylacium aduncum*, a nematode species, and *Scolex pleuronectis*, a cestode species, were reported from 38 and 29 different host species, respectively. The following most reported parasite from 24 different fish host species was an arthropod *Anilocra physodes* which has a direct life cycle. When we have a closer look at Figure 6, it can be seen that the most common digenean trematode was *Helicometra fasciata* reported from 16 different host fish species. The only ciliophoran parasite infesting the highest fish species diversity (9) was *Trichodina domerguei*. Monogeneans are generally known to strictly host species and *Microcotyle erythrini* was reported from 7 different host fish species.

32. Cultured marine fishes

Fish culture in Turkey has gained great momentum in the last 30 years and Turkey has become the leader of cultured marine fishes in Europe. The most commonly cultured marine fishes are seabass *Dicentrarchus labrax* and gilt-head sea bream *Sparus aurata* in Turkey and there are also some other alternative marine fish species that have been subjected to cultural activities. Owing to intensive cultural activities on seabass and gilt-head sea bream, the numbers of parasite species were the highest in these fish species with 22 and 11 parasite species, respectively (Figure 7). This figure also shows that the third most infected fish species is common dentex *Dentex dentex* with 10 parasite species and the rest of the cultured marine fishes have 1 to 5 parasite species.

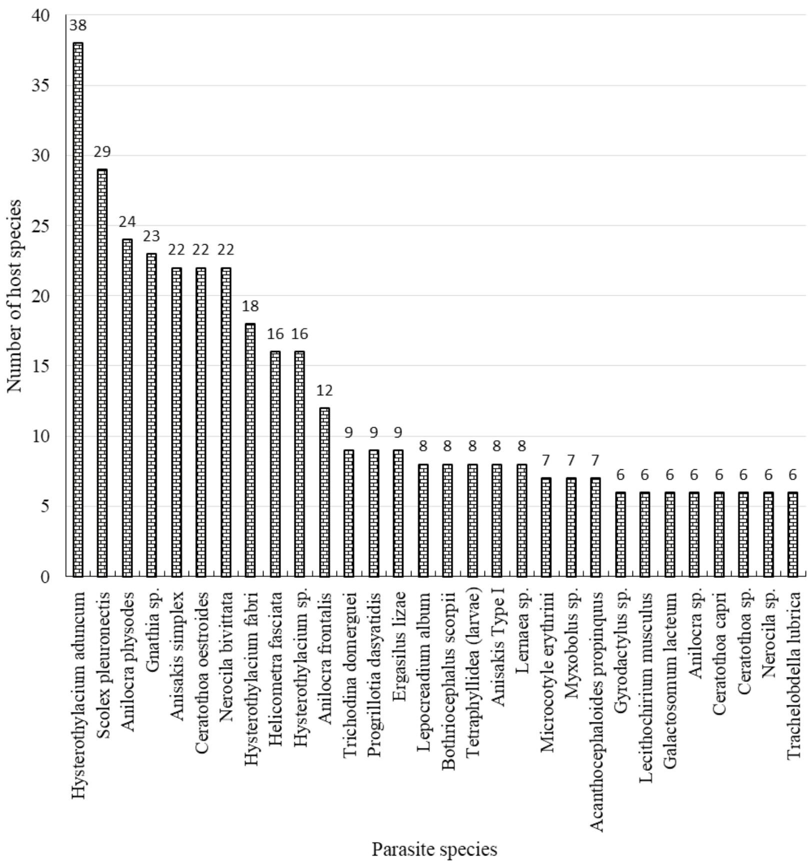

Figure 6. *The number of fish host species infected by marine fish parasites in Turkey*

Cultured marine fish subjected to parasitological investigation yielded a wide range of parasite species belonging to 7 higher taxa and the most specious is the arthropods with 19 species and followed by Monogenea and Protozoa with 10 and 9 parasite species, respectively (Figure 8). The other higher taxa had very low numbers of parasite species (Figure 8) and it must be noted that owing to controlled environments in culture facilities, no complex life cycled parasites were reported.

The number of fish host species infected by cultured marine fish parasites in Turkey is presented in Figure 9. *Ceratothoa oestroides* (Arthropoda), *Microcotyle erythrini* (Monogenea), and *Gnathia* sp. (Arthropoda) were the most commonly occurring parasites on 10, 8, and 8 cultured marine fish species, respectively. All of these parasites have only one host in their life cycle to develop and infect their host fishes. Figure 9 also shows clearly that the rest of the parasites again had basic life cycles involving only water columns and fish hosts, and among them, *Epistylis* and *Trichodina* are ciliophoran protozoan parasites that multiply by binary fission on their host fishes. Monogeneans lay eggs into water columns and bottom plants and then after hatching, new parasite individuals occur. All of these parasites can reach many numbers in a short period of time.

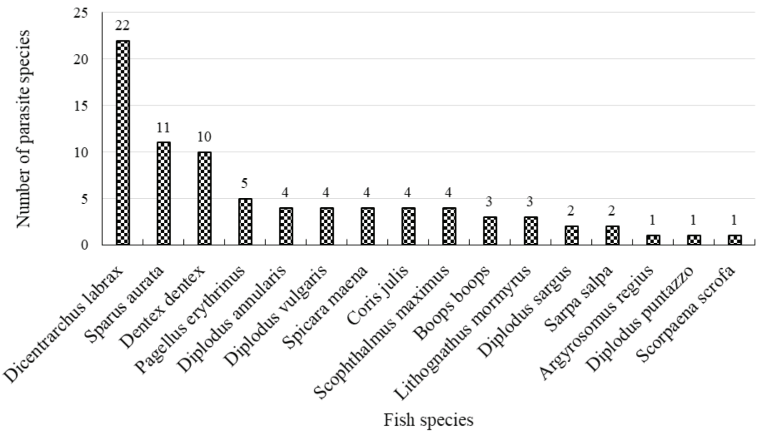

Figure 7. *The number of parasite species infecting the cultured marine fishes in Turkey*

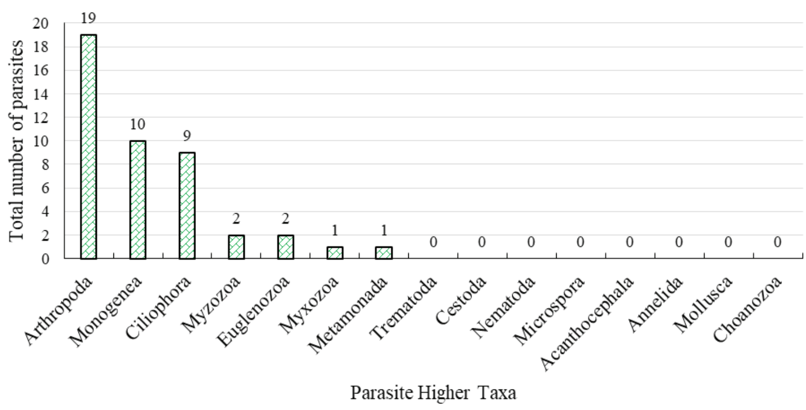

Figure 8. *The number of parasite species belonging to higher taxa infecting cultured marine fishes in Turkey*

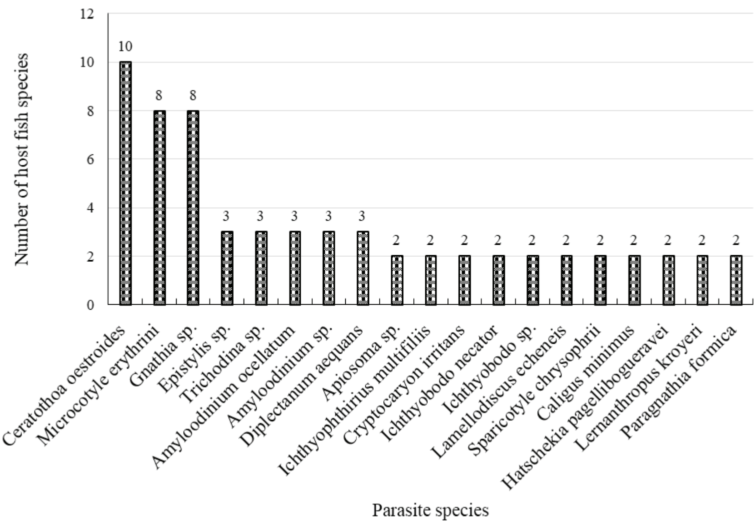

Figure 9. *The number of fish host species infected by cultured marine fish parasites in Turkey*

4. Parasite diversity of freshwater fishes

4.1.1. Wild freshwater fish

A total of 106 wild freshwater fish species were reported to be the host of at least one parasite species and among them, a cyprinid fish *Cyprinus carpio* had the highest parasite diversity of 78 species, followed by other cyprinids *Scardinius erythrophthalmus, Vimba vimba* infected by 36, 36 and 34 parasite species, respectively (Figure 10). It can be seen in Figure 10 that the following fish species had very high parasite diversity with more than 10 parasite species and the lesser number of parasite species than 7 infecting wild freshwater host fishes in Turkey can be seen in the book published by Özer (2021).

Wild freshwater fish subjected to parasitological investigation yielded a wide range of parasite species belonging to 13 higher taxa and the most specious is the monogeneans with 89 species and followed by Trematoda, Cestoda, Nematoda, and Protozoa (Figure 11). Monogeneans are among the most diverse parasitic groups of fishes and, as was mentioned above, their simple life cycle strategies allow them to reproduce in high numbers in freshwater environments. Water temperature is one of the key drivers for their reproduction and infection processes, especially in the late spring and summer seasons.

The number of fish host species infected by wild freshwater fish parasites in Turkey is presented in Figure 12 and it can be seen that the dominating parasite species was a digenean trematode *Diplostomum* sp., followed by other cestodes *L. intestinalis* and *B. acheilognathi*, an acanthocephalan *N. (N.) rutili* with the host number of 34, 32, 28, 24, respectively.

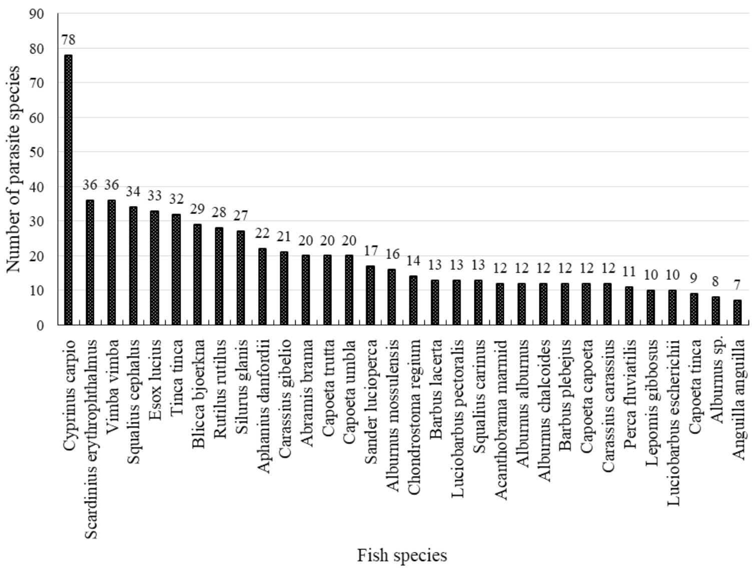

Figure 10. *The number of parasite species infecting the wild freshwater fishes in Turkey*

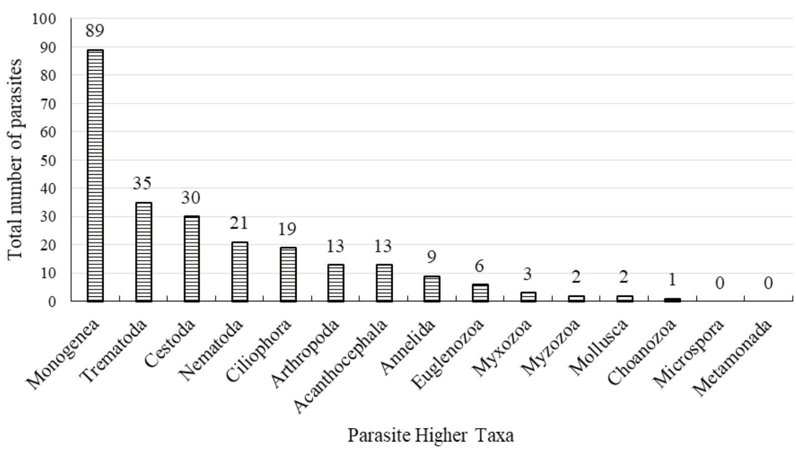

Figure 11. *The number of parasite species belonging to higher taxa infecting wild freshwater fishes in Turkey*

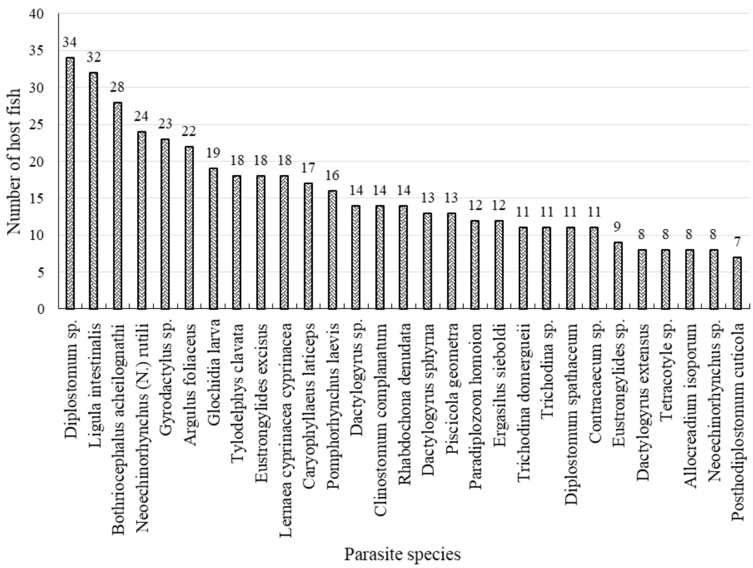

Figure 12. *The number of fish host species infected by wild freshwater fish parasites in Turkey*

4.1.2. Cultured freshwater fish

Intensive freshwater fish culture has been dominated by rainbow trout *Oncorhynchus mykiss* for decades and common carp *Cyprinus carpio* has been the second most commonly but extensively cultured species in Turkey. Owing to their dominating cultural activities, the number of parasite species infecting the cultured freshwater fishes in Turkey are 27 for rainbow trout and 11 for common carp (Figure 13). The rest of the cultured fish species had also been infected by several parasites species and the details can be seen in Figure 13.

When the number of parasite species belonging to higher taxa infecting cultured freshwater fishes in Turkey is considered, Protozoan and Monogenean higher taxa were represented by the highest numbers of parasites of 17 and 9, respectively (Figure 14). This figure also illustrates the numbers of parasites belonging to other parasitic taxa infecting cultured freshwater fishes.

Among the cultured fish infesting parasites, ciliophorans *Chilodonella cyprini, Ichthophthirius multifiliis* and *Trichodina* sp. were the first three parasite species with 6, 4, 3 host fish species (Figure 15). These parasite species are cosmopolitan ones reported from a wide range of freshwater fishes in different environments worldwide. Monogenean *Gyrodactylus* sp., and *Dactylogyrus vastator* parasites infested 3 and 2 different host fish species and the other parasites also found rarely in limited host fish species (Figure 15).

Both genera of monogeneans are also most commonly reported freshwater parasites worldwide.

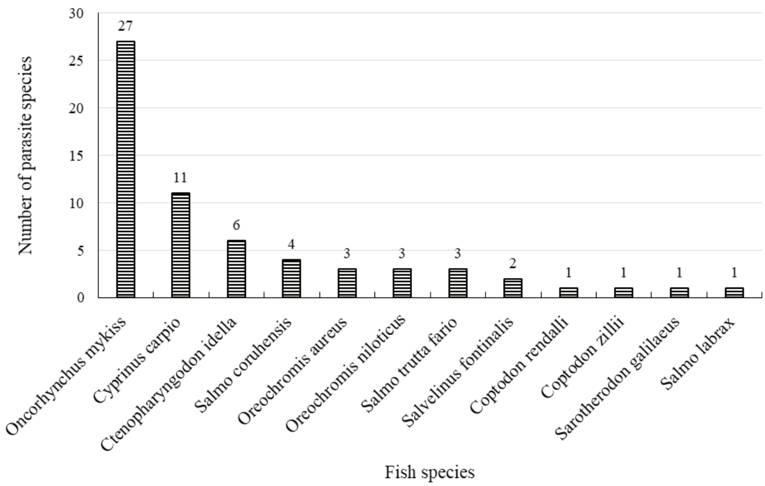

Figure 13. *The number of parasite species infecting the cultured freshwater fishes in Turkey*

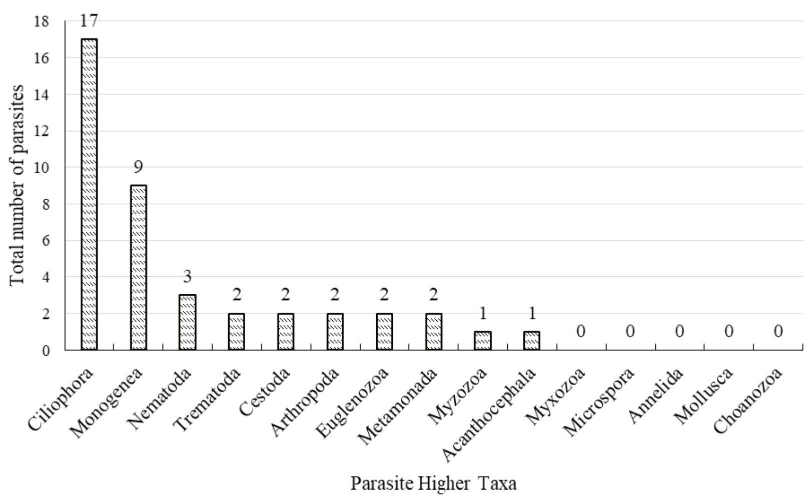

Figure 14. *The number of parasite species belonging to higher taxa infecting cultured freshwater fishes in Turkey*

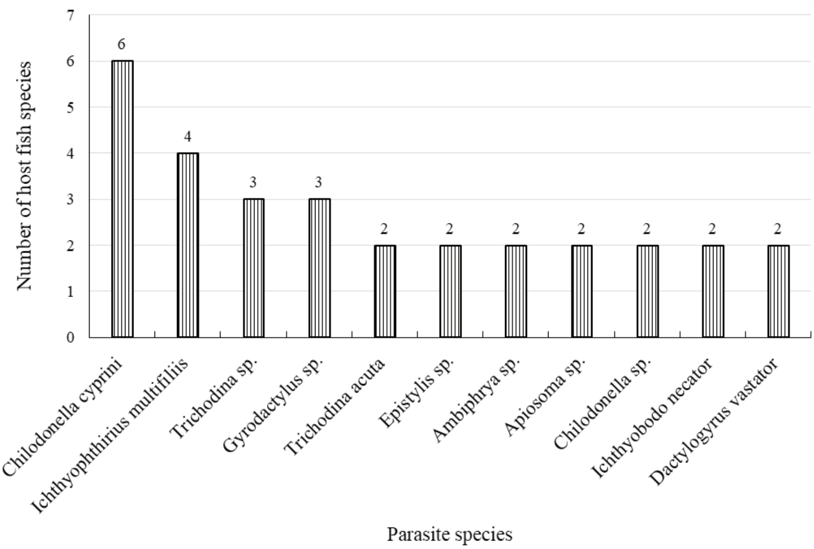

Figure 15. *The number of fish host species infected by cultured freshwater fish parasites in Turkey*

5. Parasite diversity of aquarium fishes

Aquarium fisheries are one of the worldwide popular sectors and ornamental fishes are attractive for hobbyists everywhere. Most of the ornamental fish species in Turkey are imported from abroad and/or some species are also cultured by local people or shops. A total of 32 ornamental fish species have been subjected to parasitological investigations and goldfish *Carassius auratus* has been reported to host the highest number of 19 different parasite species in Turkey (Figure 16). Guppy *Poecilia reticulata*, green swordtail *Xiphophorus helleri*, sailfin molly *Poecilia latipinna*, and southern platyfish *Xiphophorus maculatus* are the following fish hosts to be infected by the highest parasite diversity of 15, 9, 8, 8, respectively and the rest of the fish species were also reported to be parasitized by the lower number of parasites (Figure 16).

The highest number of parasite species (14) belonging to higher taxa infecting aquarium fishes in Turkey is Protozoa, followed by Monogenea (8) and Arthropoda (4) (Figure 17). Parasites belonging to these three higher taxa all have direct life cycles involving fish hosts and water columns. These parasites can multiply easily in relation to water temperature and their numbers reach very high values within a short period of time. When we have a closer look at individual parasites infecting aquarium fishes, ciliophoran *Trichodina* sp., *Ichthyophthirius multifiliis,* the agent of white spot disease, a flagellate *Ichthyobodo necator* and a dinoflagellate *Piscinoodinium pillulare* are the most dominant parasite species infecting 21, 14,9, and 6 fish species, respectively (Figure 18).

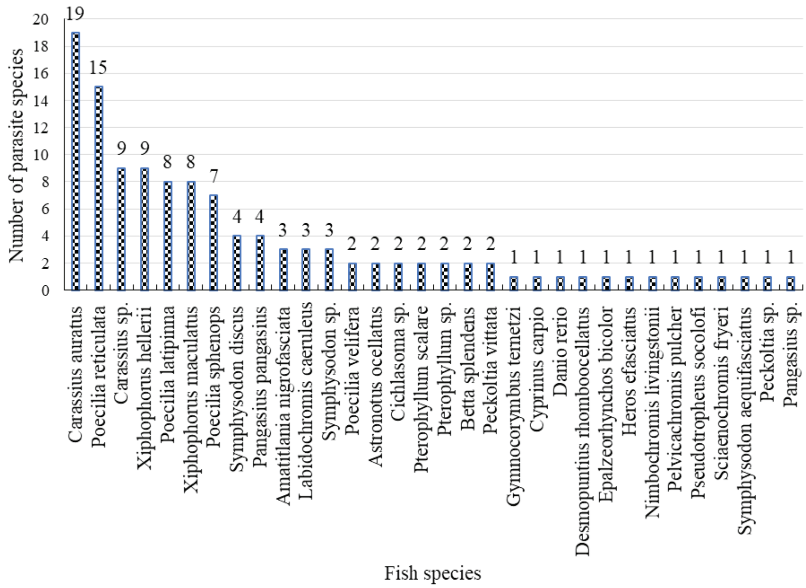

Figure 16. *The number of parasite species infecting aquarium fishes in Turkey*

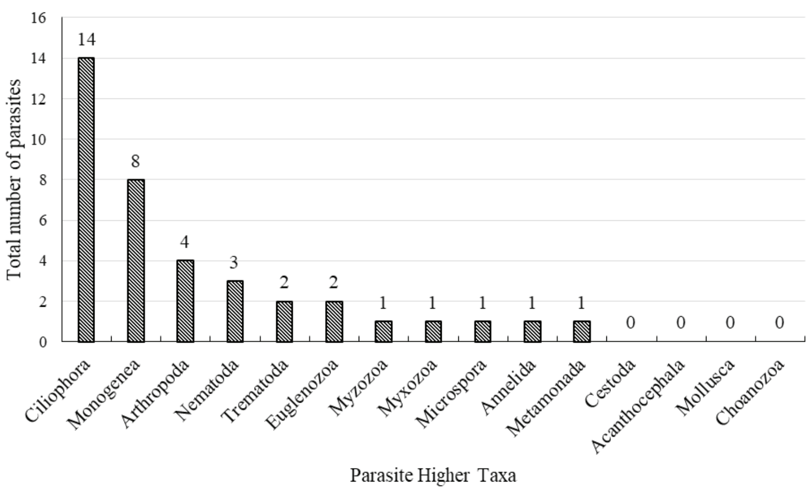

Figure 17. *The number of parasite species belonging to higher taxa infecting aquarium fishes in Turkey*

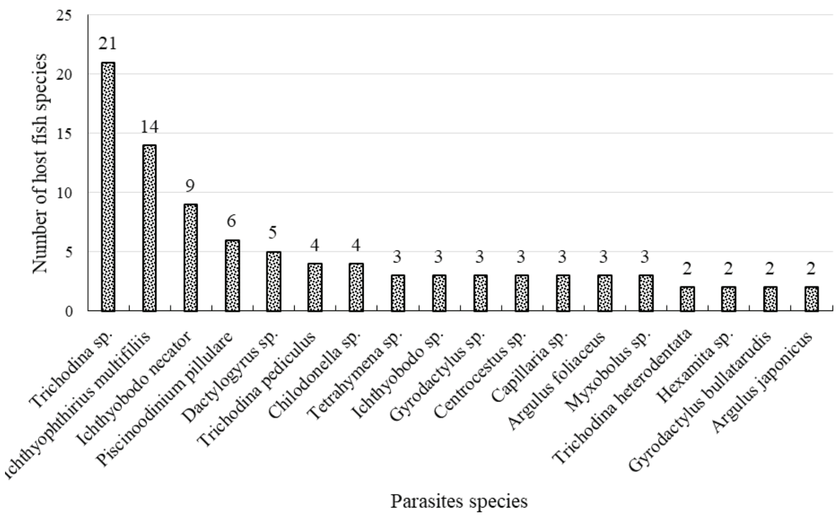

Figure 18. *The number of fish host species infected by aquarium fish parasites in Turkey*

6. Fish parasitic investigation efforts and recommendations

This chapter provided a comprehensive data for the parasites and their fish hosts in three different environments of marine, freshwater, and aquariums in Turkey and, it is clear from these data the numbers of both reported parasites species, especially in some higher taxa, and the host fish species are very low. This can be justified easily by the number of parasites reported in fish hosts when considering the reported 561 marine and 401 freshwater fish species in Turkey by Froese & Pauly (2022). On the other hand, it is not possible to provide an actual number of parasite species currently infecting their host fishes in those three environments as the results of several factors; i) the investigations focused only on the target parasite groups such as monogenea, nematoda, etc, and the neglection of some higher taxa in the investigations ii) the limited number of professionals with more comprehensive knowledge to provide investigations on the whole parasite fauna in any given fish species, iii) the financial limitations for more comprehensive countrywide research activities, iv) the limited number of facilities having more technological devices enabling more accurate parasite species identifications. We can, of course, increase these obligations on the revealing of the actual parasite compositions in investigated environments and fish hosts, but it is clear that more efforts are needed to overcome this problem. However, parasitic investigations have increased in the recent 10 – 15 years in Turkey and this provided more reports on wild and cultured fish parasites in marine, freshwater and, aquarium environments and, considering the great progress in fish culture activities in Turkey, these recent increases will provide us a better understanding of current parasitic invasions in Turkey. For example, in the recent 10 years, a great increase has occurred in the myxozoan parasites of fishes in Turkey, especially on the Black Sea coasts of Turkey, and this has yielded 4 new parasite species identification for science (see Özer 2021 for details). Similar efforts can be seen on the crustacean parasites of mostly marine fishes in the Mediterranean Sea coats of Turkey by Özak and his research teams (see Özer 2021 for details). These examples clearly show that more professionals and more research efforts will definitely provide more data on actual parasitic fauna of fishes in Turkey and, of course, more financial sources will also enable researchers to focus on more detailed research activities rather than focusing on only some parasitic groups infecting not only cultured and economically significant fish species but also wild ones in marine, freshwater and, aquarium environments in Turkey.

7. Conclusion

Turkey has a great source of water in both inland freshwater and coastal marine environments and this great potential occupies a very diverse fish species in both environments. The ornamental fish trade in our country is also another issue to be considered for fish diversity. Aquaculture activities in Turkey have gained great momentum in the recent 20 years and intensively culturing some species made Turkey one of the tops in Europe. Of course, fish species in all these sources have at least one parasite species in their lifespan and the figures provided above clearly showed that some fish species either in the wild or cultured had experienced this assumption and more investigations will reveal more parasite diversity in fish hosts in Turkey.

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