A LOOK INTO SOME RECENT ADVANCES IN BIOLOGY, ECOLOGY AND AGRICULTURAL PRACTICES

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PREFACE

Today, research in the field of life sciences is more inclined to interdisciplinary studies. Recent advances in technologies have led to a better understanding of living systems, which reduced the boundaries between sub-disciplines of the life sciences. This new trend in agricultural research includes studies that combine different disciplines such as microbiology, biotechnology, molecular biology, biochemistry, and ecology.

When designing a farm system that uses nature as a model, ensuring diversity in most ecosystems helps to sustain resilient agro-ecosystem. In plant or animal organic farming, these difficulties can be very intense and tedious especially at the beginning.

This book is written with the aim of providing up-to-date information in their fields, with a variety of approaches ranging from different articles covering biology, ecology, bioremediation, parasites, biotechnology, pathogens, bacteria, phytochemistry, phytopharmacology and flowering plants up to trade and marketing. The book has been prepared for the benefit of researchers and advanced researchers as well as young undergraduate and graduate students.

Lecturer Zubeyir GUNES

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CHAPTER 1

PLANT-ASSOCIATED MICROBIAL BIOFILMS-MEDIATED BIOREMEDIATION OF HEAVY METALS

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INTRODUCTION

The accumulation of hazardous contaminants in environments has been increasing globally due to natural disasters and anthropogenic activities such as urbanization, rapid industrialization, and agricultural practices (Ojuederie and Babalola, 2017). Toxic pollutants such as heavy metals can accumulate in soils, which in turn adversely affects plants and beneficial rhizobacteria as well as soil health. Some essential metals (i.e. copper, cobalt, iron, and zinc) are required for certain functions of organisms in small quantities but the higher amounts of essential and non-essential heavy metals lead to various physiological, biochemical, and metabolic disorders in plants, subsequently decreasing plant growth and productivity (Dalvi and Bhalerao, 2013). Removing heavy metals from soils is of utmost importance to alleviate their effects on plants and ensure food security.

Several physical, chemical and biological methods have been employed for the clean-up of toxic metal ions from soils, but among them, the use of biological techniques called bioremediation appears to be the best approach (Hassan, et al., 2017). Bioremediation is a cheap, effective, and eco-friendly biological technique, when compared to conventional methods, utilized for the removal of toxic metals from the contaminated environments using the bio-mechanisms of microbes and plants (Ojuederie and Babalola, 2017). Certain species of bacteria, fungi, and algae have potential to remove heavy metals from various environments (Choudhary, et al., 2017, Hassan, et al., 2017). The exploitation of indigenous metal-resistant microbes capable of reducing the metals is considered an essential and efficient way of eradicating metal pollutants from the ecosystem (Ojuederie and Babalola, 2017). These organisms are able to degrade toxic metals or convert them into less-toxic forms without producing toxic by-products (Hassan, et al., 2017; Ojuederie and Babalola, 2017).

Microorganisms can sequester toxic metals from their environments by the cell wall components, metal-binding peptides or proteins, and siderophore production (Ojuederie and Babalola, 2017) as well as the secretion of extracellular polymeric substances (Morcillo and Manzanera, 2021), therefore application of such microbes can alleviate metal stress in plants. The release of microbial extracellular polymeric substances, particularly exopolysaccharides, is an effective way of chelating metal cations from the surroundings by their negatively charged functional groups, ultimately restricting the excessive uptake of heavy metals by plant roots.

The majority of earlier studies have focused on the remediation potential of planktonically living microorganisms but planktonic microbes can be negatively affected by environmental stress factors. Biofilm formation offers several advantages to biofilm-forming cells such as protection against various external stresses (Flemming and Wingender, 2010), therefore recent studies pay more attention to biofilm-mediated bioremediation (Sharma, et al., 2020; Yadav and Chandra, 2020; Sharma, 2021). In a comparison with planktonic counterparts, microbial biofilms are capable of efficiently absorbing, immobilizing, and degrading a great variety of pollutants including heavy metals from a diverse range of environments, and also better adapting, surviving, and persisting under various stress conditions (Sharma, et al., 2020; Yadav and Chandra, 2020). However, studies on biofilm-mediated remediation in soils are very limited. Considering the advantageous roles of biofilms in heavy metal removal and the survival of microbes, this work aimed to show the bioremediation potential of plant-associated microorganisms through biofilm formation together with exopolysaccharide production.

The effects of heavy metals on plants

Excessive accumulation of heavy metals in agricultural soils such as zinc (Zn), cadmium (Cd), copper (Cu), mercury (Hg), chromium (Cr), lead (Pb), arsenate (As), cobalt (Co), nickel (Ni), manganese (Mn), iron (Fe), and aluminum (Al) poses major threats to plants (Nagajyoti, et al., 2010; Emamverdian, et al., 2015). Although certain heavy metals are required for plant growth and performance, the excess availability of such ions results in several physiological, morphological, biochemical, and metabolic disorders in plants. When the amounts of such specific metals in plants exceed the optimal levels, unless inactivated or tolerated, they may directly or indirectly affect plant growth and productivity. Decreased cytoplasmic enzyme activities and damaged cell structures are among the direct toxic effects (Chibuike and Obiora, 2014). A good example of an indirect effect is the disappearance and reduced activity of beneficial soil microbiome. Elevated metal concentrations adversely affect the existence of beneficial soil microorganisms, which in turn decreases the decomposition of soil organic matter, ultimately decreasing the levels of essential nutrients (Chibuike and Obiora, 2014). It is well-established that beneficial soil microbes decompose organic compounds into available forms, so are of utmost importance for soil productivity (Yan, et al., 2015; Çam, 2022a).

In general, plants exposed to high concentrations of metals decrease their growth and development, interrupting metabolic and photosynthetic pathways, and reducing biomass production. For example, zinc and cadmium toxicity interferes with plant metabolic functions, affecting the catalytic efficiency of enzymes, causing oxidative damage, and limiting plant growth and productivity (Nagajyoti, et al., 2010). The phytotoxicity of zinc also decreases root and shoot growth, causing early senescence, leading to chlorosis and purplish-red color in plant leaves (Nagajyoti, et al., 2010). The toxicity of Cd causes poor seed germination, reduced plant nutrient content, and inhibition of root and shoot growth in different plants (Chibuike and Obiora, 2014). Similarly, a higher concentration of copper reduces plant yield and growth, impeding seed germination and chlorophyll biosynthesis, causing stunted leaf and root growth, limiting the uptake of essential elements, and leading to the production of reactive oxygen species (ROS) (Adrees, et al., 2015). In a study, the detrimental effect of Cu toxicity was well-investigated. It was observed that the root length of maize treated with 78.7 and 157 μM copper is reduced by up 100% compared to untreated control groups while a 90.6% reduction was reported in root length exposed to 15.7 μM copper treatment (Ait Ali, et al., 2002). Another important metal ion is mercury because its excess amount affects mitochondrial activity, causing ROS-induced oxidative stress, therefore disrupting membrane lipids and cellular metabolism (Nagajyoti, et al., 2010).

Excess of chromium impedes stem and leaf development, inhibiting cell division and root elongation, restricting water and nutrient uptake, reducing dry matter production, decreasing amylase activity and sugar levels, and interfering with the uptake of various essential nutrients (Fe, K, Ca, Mg, Mn, and P) in different parts of plants (Emamverdian, et al., 2015). Treatment of varying concentrations of Cr in sugarcane revealed a 32–57% reduction in the germination between 20 and 80 ppm and a 95% reduction in root length at 40-80 ppm with respect to non-treated controls (Radha, et al., 2000). Leaf chlorosis and necroses were also observed at 40 and 80 ppm Cr, respectively. The phytotoxicity of lead, one of the most abundant toxic metals, inhibits seed germination, root and/or stem elongation, and leaf expansion; causes chlorosis and a significant reduction in the biomass production; obstructs enzyme activities; affects photosynthesis through the inhibition of carboxylating enzymes; disrupts water and mineral balance; alters cell membrane permeability; leads to oxidative stress by triggering ROS production (Nagajyoti, et al., 2010). Likewise, arsenic accumulation in soils reduces plant growth and reproductive capacity by interfering with important metabolic processes and damages cellular membranes, causing electrolyte leakage (Finnegan and Chen, 2012). Phytotoxicity experiments of cobalt in barley, oilseed rape, and tomato in different types of soils demonstrated a significant reduction (over 50%) in plant shoot biomass (Li, et al., 2009). Higher concentration of cobalt also affects the levels of most essential nutrients such as P, Mn, Fe, and Zn and markedly reduces water potential and transpiration rate in cauliflower (Chatterjee and Chatterjee, 2000).

Accumulation of excessive nickel in the soil causes chlorosis and necrosis in plants, impairing nutrient and water balance, disturbing membrane functionality and ionic balance, and affecting lipid content and H-ATPase activity of cell membranes (Nagajyoti, et al., 2010). The toxicity of Ni also results in a reduction in chlorophyll content, plant nutrient acquisition, shoot yield and root growth, and stomatal conductance (Chibuike and Obiora, 2014). Similarly, high level of manganese triggers oxidative stress, decreasing enzyme activity, inhibiting chlorophyll synthesis and photosynthesis, and disrupting the absorption and translocation of micro- and macro-nutrients such as Fe, Mg, and P; consequently causing chlorosis and necrotic dark spots in plant leaves, finally limiting plant growth and production (Li, et al., 2019). Excess iron disturbs plant metabolism by affecting the entry and transportation of major elements (Zaid, et al., 2020). Iron toxicity also results in the production of free radicals, which in turn impairs cellular structure, damaging cell membranes, nucleic acids, and proteins, ultimately reducing plant photosynthesis and yield (Nagajyoti, et al., 2010). Aluminum is another important inhibitory element for plant growth and development. The phytotoxicity of Al causes a reduction in root respiration, disturbance in the regulation of sugar phosphorylation, hindrance of cell division in plant roots, damage in aerial parts of plants, small necrotic spots and chlorosis in leaves, reduction in stomatal aperture and photosynthetic activity, and perturbation in the uptake and translocation of some essential nutrients such as Mg, K, and Ca (Emamverdian, et al., 2015).

Plant defense strategies

In order to maintain metal homeostasis and confer tolerance to metal stress, plants have developed various strategies, particularly in metal-enriched soils. Certain plants can avoid and tolerate metal stress by a variety of defense mechanisms such as the secretion of root exudates, metal-immobilizing properties of cell wall, active efflux pumping of metal ions in plasma membrane, sequestration of metals in the vacuoles, detoxification of toxic metals through organic acids, inactivation of metals through phytochelatins and metallothioneins, upregulation of stress-related proteins, activation of metal-responsive genes, production of reactive oxygen species, synthesis of signaling molecules, hormone biosynthesis, and symbiotic associations with mycorrhiza (Manara, 2012; Dalvi and Bhalerao, 2013). Through these defense strategies, plants restrict the uptake of metals and inhibit their entry and accumulation into tissues, if achieved to enter into plant tissues, tolerate them through inactivation, detoxification or conversion into less toxic forms (Dalvi and Bhalerao, 2013).

Microbial mechanisms in the mitigation of metal stress

Apart from plant defense strategies, microorganisms can alleviate heavy metal stress in plants through some direct and indirect mechanisms. Organisms such as bacteria, fungi, and algae have been used for the clean-up of heavy metals from polluted environments (Choudhary, et al., 2017; Hassan, et al., 2017). These microorganisms are capable of degrading toxic ions or converting into non-dangerous forms (Ojuederie and Babalola, 2017) without producing toxic byproducts (Hassan, et al., 2017).

Microorganisms can remove heavy metals through a process called biosorption. The metal ions can be captured or absorbed in the cellular structures. This is called biosorption (Hassan, et al., 2017). Biosorption mechanisms include transport across membranes, precipitation, complexation, ion exchange, and physical adsorption (Ojuederie and Babalola, 2017). Extracellular polymeric substances and cell wall properties of microbial cells play a crucial role in metal adsorption through precipitation and proton exchange due to their high metal-binding potential (Verma and Kuila, 2019). Microbes have the ability to sequester toxic ions through their cell wall components, metal-binding peptides or proteins, and siderophore production (Ojuederie and Babalola, 2017). For example, the charged functional groups such as carbonyl, phosphate, and hydroxyl groups in the surface of bacterial cell walls have potential to chelate metals from surroundings (Ojuederie and Babalola, 2017), thus making toxic metals less accessible for plants. The components of bacterial cell walls including amino acids, proteins, lipopolysaccharides, teichoic acids, and others act as ligands for chelating heavy metals, ultimately contributing to metal remediation from polluted sites (Ojuederie and Babalola, 2017). Besides, fungi act as effective biosorbents by absorbing toxic metals in their mycelium and spores and detoxifying them through accumulation, intra- and extra-cellular precipitation, and transformation (Ojuederie and Babalola, 2017). Similar to other microorganisms, algae can also remediate heavy metals through two main mechanisms (biosorption using non-living cellular components and accumulation using living cells) (Hassan, et al., 2017; Sen Gupta, et al., 2020).

Moreover, siderophores have a strong affinity for heavy metals, being able to chelate the toxic ions from their environments, thus playing a critical role in heavy metal clearance (Schalk, et al., 2011). Microbes also remediate soils from metal pollutants by the conversion of metal ions into harmless forms through redox processes. Redox reactions chemically convert toxic metals in soil into innocuous or less toxic forms which are less mobile and more stable (Ojuederie and Babalola, 2017).

The potential of microorganisms on plant heavy metal tolerance

Using one or more of all the mechanisms mentioned above, microbes restrict a higher metal accumulation and alter the bioavailability of toxic metal ions in plants, therefore indirectly contributing to plant growth. A great number of microorganisms have been widely used for the bioremediation of metals in soils as well as the mitigation of metal toxicity in plants. The exploitation of certain microorganisms for the remediation of metal-abundant soils is considered a very effective, cheap, and safe approach in metal clean-up procedures (Hassan, et al., 2017; Verma and Kuila, 2019), therefore has received more attention recently. For instance, the inoculation of arsenate-reducing bacteria increased the remediation efficiency of arsenic-enriched soils and the biomass of *Pteris vittata* L. by 53%, and reduced arsenic leaching from 21 to 71% under arsenic-polluted soil conditions (Yang, et al., 2012). The examination of metal-tolerant *Bacillus thuringiensis* strain OSM29 at varying concentrations of heavy metals (Cr, Cd, Pb, Cu, and Ni) revealed the metal-removing potential of this strain in cauliflower rhizosphere (Oves, et al., 2013). They also observed that the biosorption capacity of the strain varies depending on the type and concentrations of metals as well as pH. The highest biosorption capacity was found in nickel by 94%.

Application of endophytes has been considered a promising approach for the cleanup of metal-polluted soils (Chen, et al., 2014; Ma, et al., 2015; Hassan, et al., 2017). In a study, it was reported that growth-promoting endophytic bacteria have great potential in improving heavy metal phytoremediation (Ma, et al., 2011). Inoculation of one of endophytic bacteria, *P. koreensis* strain AGB-1, enhanced heavy metal solubilization and tolerance, enzyme activities, the plant biomass by 54%, and chlorophyll content by 27%, suggesting the potential of this strain in remediation of soils amended with Cd, As, Pb, Zn, and Cu (Babu, et al., 2015). Likewise, the treatment of endophytic *Rahnella* sp. JN6 in soils amended with different levels of Cd, Zn, and Pb increased the dry weights of rape plants and metal tolerance, indicating the importance of the strain in phytoremediation (He, et al., 2013). Similarly, inoculation of metal-resistant endophytic strains of *Bacillus*, *Achromobacter*, and *Stenotrophomonas* spp. isolated from the tissues of hyperaccumulator *Sedum plumbizincicola* showed the potential of microbes in improving plant growth parameters and increasing phytoextraction capacity of soils contaminated with Cd, Zn, and Pb (Ma, et al., 2015). Another endophytic bacterium *Sphingomonas* strain SaMR12 increased plant growth and cadmium tolerance in plants by regulating the secretion of organic acids and inhibiting plant oxidative stress (Chen, et al., 2014).

In addition to bacteria, fungi and microalgae have been employed for the mitigation of heavy metal stress. Colonization of plants by arbuscular mycorrhizal fungi in metal-polluted soils showed their potential in heavy metal tolerance (Hildebrandt, et al., 2007). Arbuscular mycorrhizal inoculation with *Glomus mosseae* markedly restricted cadmium and lead uptake into plant roots and transportation of the metals into the upper parts of plants (Garg and Aggarwal, 2012). In another work, fungal inoculation in mycorrhizal maize seedlings decreased the phytotoxicity of lead through the cell wall of the fungi (Zhang, et al., 2010). Inoculation of mycorrhizal fungal isolates in sunflower plants enhanced plant growth and biomass by reducing the effect of metal stresses (Hassan, et al., 2013). They also reported that the fungus *Rhizophagus irregularis* increases cadmium phytoextraction, while *Funneliformis mosseae* enhances the phytostabilization of cadmium and zinc in metal-contaminated soils. Achal et al. (2011) showed the high potential of the fungus *Gloeophyllum sepiarium* in the bioremediation of chromium-enriched soils. This brown-rot fungus exhibited a 94% reduction in chromium concentrations in soils by its biomass in the period of six months (Achal, et al., 2011). Furthermore, microalgae species have huge potential in the alleviation of metal toxicity. Several microalgae isolates have been utilized for the removal of various toxic metals from metal-contaminated environments (Aksu and Dönmez, 2006; Hameed and Ebrahim, 2007; Monteiro, et al., 2010; Shanab, et al., 2012; Hassan, et al., 2017; Sen Gupta, et al., 2020), but the studies of microalgae application to plants are rare in the literature.

Combined application of metal-reducing microorganisms rather than sole treatments has been suggested for effective bioremediation of toxic metals. The examination of combined or sole inoculation of different bacterial cultures for the remediation of lead, cadmium, and copper metals revealed that the application of bacterial mixtures is found to be more effective compared to that of single strain culture (Kang, et al., 2016). The highest bioremediation efficiency was recorded for lead by 98.3%. Likewise, combinations of bacteria with fungi exhibit better efficiency for the removal of heavy metals. For instance, co-inoculation of the mushroom *Agrocybe aegerita* with the bacterium *Serratia* spp. more successfully remediated nickel and cadmium toxicity in soils with respect to sole inoculation (Li, et al., 2016). It was well-discussed by Nadeem et al. (2014) that the synergistic interactions between PGPR and mycorrhizal fungi might contribute to the achievement of maximum benefits under stressful conditions.

Potential of EPS in the adsorption of heavy metals

Aside from the strategies discussed in the section ‘‘microbial mechanisms in the mitigation of metal stresses’’, the most important microbial action in the sequestration of heavy metals is the production of exopolysaccharide (EPS). Microbial EPS is the high molecular weight polymers secreted by microbes in response to several stresses, protecting EPS-producing organisms and plants from the detrimental effects of external stresses including metal toxicity (Morcillo and Manzanera, 2021). The underlying mechanism of metal remediation through microbial EPS is its metal-binding properties. Metal-sequestration capability of EPS helps in the biosorption of metal ions. EPS composition is abundant in anionic moieties that increase the remediation of heavy metals, particularly cationic ones, from the surroundings (Ojuederie and Babalola, 2017). Non-specific electrostatic interactions take place between positively charged metals and negatively charged functional residues of EPS, which results in the formation of stable complexes (Pal and Paul, 2008). The occurrence of these EPS-mediated complexations is associated with the absorbance of toxic metals from soils, ultimately limiting the excessive uptake of toxic metals by plants.

The direct role of microbial EPS in metal remediation has been shown in several investigations. In a most recent study, the metal adsorption capabilities of microbial exopolysaccharides were well-shown under *in vivo* and *in vitro* conditions. EPS-producing rhizobacterium *Halomonas* sp. strain Exo1 increased rice growth under arsenic stress in pot assays most likely through EPS production (Mukherjee, et al., 2019). They suggested that arsenic metals stimulate EPS production, which in turn sequesters As through its substrate-binding potential, indicating a positive feed-back mechanism. Apart from this study, a great number of studies have focused mostly on the bio-removal of toxic metals from aqueous solutions under *in vitro* conditions. The utilization of EPS in the clean-up of metal ions from soils is very rare in literature; therefore, in this section, the potential use of EPS will be explained with a focus on the biosorption of metals from aqueous solutions by a wide range of microbes. For example, EPS produced by *Bacillus firmus* strain ME-102 was non-specifically able to chelate zinc, copper, and lead metals from aqueous solution with a ratio of 61.8%, 74.9%, and 98.3%, respectively (Salehizadeh and Shojaosadati, 2003). Similarly, EPS secreted by the strain XU1 of *Azotobacter chroococcum* sequestered lead and mercury metals with an adsorption capacity of approximately 40% and 48%, respectively (Rasulov, et al., 2013).

EPS-producing *Enterobacter cloaceae* strain adsorbed about 75% of chromium ions from a 100 ppm Cr-containing seawater through both cell pellet and EPS, suggesting the potential use of this microorganism in chromium bioremediation (Iyer, et al., 2004). In that study, EPS was considered the primary contributor to chromium removal compared to bacterial pellet. Supportingly, the great contribution of EPS was also observed in another study conducted with *Ensifer meliloti* strain MS-125. Comparison of binding-capacities of exopolysaccharides and dried cells revealed the superiority of EPS in metal chelation. The adsorption rates of EPS and dried cells for Zn, Ni, and Pb from the solutions were 66-89% and 44-73%, respectively (Lakzian, et al., 2008). Many other microorganisms also accumulated metal ions through their exopolysaccharides such as arsenic by *Herminiimonas arsenicoxydans* (Muller, et al., 2007); copper and lead by *M. organophilum* (Kim, et al., 1996); zinc and lead by *Shewenella oneidensis* (Ha, et al., 2010); manganese by *Anabaena spiroides* (Freire-Nordi, et al., 2005); lead by *Gloeocapsa gelatinosa* (Raungsomboon, et al., 2006), *Calothrix marchica* (Ruangsomboon, et al., 2007) and *Paenibacillus jamilae* (Morillo, et al., 2006; Morillo Pérez, et al., 2008); copper by *Cyanospira capsulate* (De Philippis, et al., 2007) and *Paenibacillus polymyxa* (Prado Acosta, et al., 2005).

In addition to homogenous consortial EPS, the utilization of mixed culture EPS appears to be effective in the biosorption of heavy metals. In a study, it was reported that the adsorption efficiency of EPS produced by a group of microorganisms was between 67 and 78% for different metals, indicating the profound impact of heterogeneous consortial EPS in metal removal (Gawali Ashruta, et al., 2014). The obtained extracellular substances from waste sludge mixed cultures instead of sole culture removed over 90% of heavy metals (Zn, Cu, and Cr) from 10-100 mg L^{-1} metal solutions, suggesting the feasibility of mixed culture EPS in metal chelation (Liu, et al., 2001). In a more recent work, the removal capacity of EPS from mixed cultures was found to be greater in media with molasses with an efficiency rate of 32% for Ni, 51.2% for Cr, and 75.7% for Cu (Kiliç, et al., 2015).

The chelating properties of EPS underlie its structure and content. The carbohydrate and non-carbohydrate substituents such as various carboxyl, sulfhydryl, amine, and hydroxyl groups in biochemical composition of EPS are responsible for the negative charge (Morcillo and Manzanera, 2021). The anionic groups in EPS interact with metal cations, creating electrostatic interactions between them, ultimately leading to the biosorption of toxic metal ions. For instance, the metal-binding potential of exopolysaccharide produced by *P. jamilae* was attributed to the presence of uronic acids whose negative charged groups interact with divalent cations (Morillo Pérez, et al., 2008). In this study, the EPS sugar content was mainly composed of glucose and mannose. They also observed that the EPS affinity for lead was ten-fold greater compared to the other metals, suggesting that the specificity and metal-sequestration capacity of microbial EPS vary by different metals depending on its functional groups.

EPS sugar substituents and their amounts greatly differ in different microorganisms depending on many factors such as culture conditions and different growth phases (i.e. log, lag, and stationary phase) (Raungsomboon, et al., 2006, 2007). The abundance and limitation in nutritional status, microbial species, type and composition of substrate, exposure to toxic substances, C/N ratio, and environmental conditions are among the factors which greatly impact EPS production and composition (Pal and Paul, 2008). For example, the presence of cadmium metals changes the conformation of exopolysaccharides produced by rhizosphere bacteria to reduce the effect of metal toxicity on cells (Kowalkowski, et al., 2019). EPS production by a rhizobacterial strain progressively increased with increasing concentrations of chromium by up to 200 µg mL^{-1} but decreased at 250 µg mL^{-1} (Karthik, et al., 2017). Likewise, EPS production was gradually enhanced up to 6 mM concentrations of arsenic but significantly reduced at 8 mM (Mukherjee, et al., 2019). These studies indicate that metal ion concentrations can enhance EPS production to some extent. Salinity is another important factor greatly affecting EPS composition and yield (Çam, 2022a). Mukherjee et al. (2019) showed that EPS production regularly increases up to 20% salt concentration. However, a sudden decline in EPS yield was observed when salt concentration was 22.5%. Temperature is also a determining factor in EPS production by plant-associated microorganisms (Morcillo and Manzanera, 2021). Additionally, the adsorption capacity of EPS for metals highly depends on pH levels. In a work, it was observed that the adsorption efficiency of bacterial EPS for lead and mercury is remarkably affected by pH values and markedly increased under acidic conditions by up to approximately 41 and 48%, respectively (Rasulov, et al., 2013). This may be due to the impact of pH on the activity of functional residues of EPS and their competition for specific metals (Salehizadeh and Shojaosadati, 2003). All the factors causing changes in EPS production, structure, and contents alter the metal-binding capacity of EPS, which in turn affects the alleviation of metal-induced stress by EPS-producing microorganisms in a positive or negative manner.

The advantages of biofilm formation

Biofilms can be simply described as an organized cluster of microbes encapsulated in a self-generated exopolymeric matrix and attached to various abiotic and biotic surfaces. Biofilms can be formed by a single- or mixed-species of bacteria, fungi, or algae. Biofilm-forming microorganisms can be phenotypically and metabolically distinct from their free-living homologues (Flemming and Wingender, 2010; Velmourougane, et al., 2017) due to the changes in transcriptional regulation of genes (Çam and Brinkmeyer, 2020a). Biofilm formation protects biofilm cells from several unfavorable conditions such as nutrient deficiency, salinity, UV exposure, antimicrobial compounds, drought stress, toxic chemicals and heavy metals, shear stress, solvents, and pH changes (Jefferson, 2004; Sharma, et al., 2020; Morcillo and Manzanera, 2021), therefore having been considered a selective survival strategy of microbes in natural environments (Çam and Brinkmeyer, 2020b). Biofilm mode of life increases the survival ability of microbes under adverse conditions, thus microorganisms tend to reside in biofilm in nature (Çam, 2022b).

Dry mass of most biofilms consists of microorganisms (<10%) and extracellular matrix (>90%) (Flemming and Wingender, 2010). Biofilm matrix is primarily composed of proteins, lipids, extracellular DNA, and exopolysaccharides. Polysaccharides are the main fraction of biofilm matrix because mutant strains lacking EPS-synthesizing genes cannot establish three-dimensional mature biofilms (Flemming and Wingender, 2010). This matrix encloses biofilm-producing cells and protects them against a wide range of environmental stresses including metal toxicity due to its protective mechanisms. The reasons why microorganisms prefer biofilm mode of growth in natural environments may be associated with the following functions of biofilm matrix as summarized by Flemming and Wingender (2010). First of all, biofilm matrix acts as a physical protective barrier against various antimicrobial substances (i.e. disinfectants and antibiotics) and toxic pollutants such as heavy metals. That is, the biofilm matrix impedes the penetration of these kinds of compounds into biofilm cells, subsequently protecting microorganisms from the negative effects of such agents. Secondly, the matrix creates a hydrated layer around biofilm-forming microbes through hydrophilic polysaccharides, thus increasing their tolerance to desiccation under water-limiting conditions. One of the most important advantages of biofilm formation is the accumulation of organic compounds (i.e. C, N, P, etc.) from the surroundings through the sorption capability of the matrix. With this ability, the matrix provides a nutrient-rich environment for biofilm communities. In addition, this matrix serves as a digestive system by metabolizing dissolved biopolymers in biofilms through extracellular enzyme activity. These metabolized nutrients can be used as energy and nutrient source. The matrix also keeps biofilm-forming cells in close proximity, therefore facilitating the interactions between the cells such as cell-cell communication and horizontal gene transfer (Flemming and Wingender, 2010). In nature, biofilm-producing microorganisms appear to be much less vulnerable to habitat-imposed natural environmental conditions than their free-living counterparts; therefore, plant-associated microbes are expected to switch from planktonic life forms to biofilm mode of life to maintain their survival under stringent environmental conditions.

Biofilm-mediated metal bioremediation

Biofilm-mediated bioremediation has been considered an ecologically friendly and cost-effective strategy for the biological remediation of pollutants (Sharma, et al., 2020). Several studies have demonstrated that microbial biofilms play a more important role in the clean-up of various hazardous pollutants (heavy metals, polycyclic aromatic hydrocarbons, pesticides, oil spills, xenobiotics, radioactive substances, etc.) from different contaminated environments (Singh, et al., 2006; Sharma, et al., 2020; Yadav and Chandra, 2020) due to their higher microbial mass and capability to scavenge several pollutants (Nilanjana, et al., 2012). For example, mixed culture bacterial biofilms are found to be suitable for the reduction of the metal chromium into less toxic insoluble forms, suggesting their potential in the bioremediation of wastewaters and soils (Smith and Gadd, 2000). Likewise, *P. aeruginosa* and *Burkholderia cepacia* biofilms are involved in the sequestration of heavy metals (Templeton, et al., 2003; Kang, et al., 2006). Another study showed the importance of bacterial biofilms in the entrapment of precipitated copper (White and Gadd, 2000). The high metal sorption capacity of biofilms in biological remediation of synthetic wastewaters contaminated with heavy metals was also demonstrated using a rotating biological contactor (Costley and Wallis, 2001). Similarly, the effective removal of metal ions and other contaminants by microbial biofilms using a bioreactor was investigated in other studies (Diels, et al., 1995; Scott, et al., 1995; Scott and Karanjkar, 1998; Travieso, et al., 2002; Von Canstein, et al., 2002; Diels, et al., 2003; Chang, et al., 2006).

In addition to the detoxification of different types of pollutants from different sources, the use of biofilm-forming plant-associated microorganisms can also be efficiently used for the bioremediation of heavy metals and other pollutants in soils. Biofilms are greatly associated with plants and have a better contribution to plant growth compared to their planktonic counterparts (Angus and Hirsch, 2013; Ahmad, et al., 2017; Çam, 2021). Plant-associated biofilms provide several functions such as protection against stress factors, decreasing microbial competition, and contributing to host plants by increasing plant growth and productivity (Kalam, et al., 2017). However, the fact that biofilm formation contributes to plant growth by mitigating the inhibitory impact of toxic metals has been shown only in very few studies. For instance, in a study, *Paenibacillus lentimorbus* biofilms increased chickpea growth and yield under Cr-amended soils (Khan, et al., 2012). This study suggested that the biofilm matrix protects *Paenibacillus* cells and plant tissues from chromium toxicity by acting as a diffusion barrier; as a result, resulting in decreasing chromium entry into plants as well as enabling bacterial cells to exert their plant growth-promoting (PGP) potential. In a more recent study, inoculation of arsenic-resistant biofilm-forming rhizobacterial strains increased the growth of rice seedlings and restricted metal uptake by plant roots (Mallick, et al., 2018). They believed that biofilm formation together with EPS production decreased arsenic accumulation in plants under natural environments.

The significance of biofilms for metal remediation-associated microorganisms

Biofilm mode of life enhances microbial survival in the rhizosphere under harsh environments (Angus and Hirsch, 2013), suggesting that stressful life in soils might be mitigated through biofilm formation. Microbial community under severely polluted sites tends to primarily exist in biofilm lifestyle to cope with stressful conditions and also for better protection and persistence (Yadav and Chandra, 2020). Biofilm-forming microbes have a greater potential to adapt and survive when exposed to adverse conditions compared to free planktonic cells and also are more resistant to stresses (Sharma, et al., 2020). For example, PGPR *Pseudomonas* produced more robust and thicker biofilms in response to zinc and lead stress, indicating that biofilm formation provides a favorable niche for microbes under stress conditions (Meliani and Bensoltane, 2016). The ability to form biofilms by *Thiomonas* sp. was considerably altered in response to arsenite exposure, causing an enhanced resistance against arsenic-induced stress (Marchal, et al., 2011). They speculated that rapid biofilm formation allows the bacteria to survive and persist under metal stress.

Apart from the inhibitory effect of metals on microbial survival, they also negatively affect PGP potential. Auxin synthesis in *Streptomyces* species was adversely affected by the presence of metals (Dimkpa, et al., 2008), suggesting that metal chelation enhances growth promotions, which in turn increases the phytoremediation of metal-exposed plants. Similarly, increasing concentrations of chromium progressively decreased PGP activities and increased EPS production to some extent in rhizobacterial strain AR6 (Karthik, et al., 2017). Maximum toxicity in PGP potential was observed at the highest concentrations of the metal. Karthik et al. (2017) suggested that the enhanced EPS production may be due to the innate protection mechanisms in response to increasing metal toxicity. An increase in the production of EPS may stimulate biofilm formation as in the case of salt stress (Çam, 2022a), which in turn protects biofilm cells from the toxic effect of chromium.

The metal-sequestering and cell-protective property of biofilms is because of the presence of EPS together with other extracellular substances

(Flemming and Wingender, 2010). EPS has great potential to sequester pollutants from surroundings but its protective effect on microbial cells is to some extent. EPS-defective *Sinorhizobium meliloti* strains were found to be very sensitive to mercury and arsenic metals, highlighting the necessity of EPS rather than biofilm formation for protection against metal stress (Nocelli, et al., 2016). On the other hand, another study revealed that Cd toxicity is alleviated through biofilm formation rather than EPS production in *Rhizobium alamii* (Schue, et al., 2011). They concluded that EPS production is not the only way protecting the cells. Transition from planktonic to biofilm lifestyle and changing bacterial metabolisms in response to metal exposure are the other factors that facilitate microbial adaptation to metal toxicity.

Soil bioremediation requires highly-adapted microorganisms due to soil heterogeneity and the toxic effect of metals on microbes (Tabak, et al., 2005; Hassan, et al., 2017). Microbial adaptation to environmental stress conditions is critical for the successful interactions and long-term persistence in plants (Çam, 2022b) as well as the efficiency of biofilm-mediated bioremediation (Yadav and Chandra, 2020). Unless microorganisms can permanently colonize the plant surfaces, they may not properly exhibit their bioremediation potential. Biofilm matrix facilitates the colonization of living and non-living surfaces by free-living cells (Flemming and Wingender, 2010). It is well-known that biofilms significantly contribute to the plant root and phyllosphere colonization of microbes (Angus and Hirsch, 2013; Çam, 2022b; Fessia, et al., 2022). In this direction, biofilm formation appears to be required for the colonization of microorganisms to plant surfaces.

Biofilm formation also increases the metal adsorption efficiency compared to planktonically living organisms. For instance, the metal-chelating capacity of biofilm cells is found to be remarkably higher than that of their planktonic counterparts (Langley and Beveridge, 1999). Briefly, in biofilm mode of growth, microbes can effectively colonize the plant surfaces and also increase their survival under changing environmental parameters as well as metal adsorption capacity. In this respect, biofilm formation along with EPS production can successfully chelate metal ions from environments, therefore protecting not only the microbial community in biofilms but also plant tissues by preventing the penetration of toxic ions into the cells, ultimately promoting plant growth and development by alleviating metal toxicity.

CONCLUSIONS

Toxic metal pollution is increasing day by day in soils worldwide, which decreases plant growth and productivity by interrupting plant metabolic and photosynthetic pathways, ultimately becoming a serious threat to food security. Application of plant-associated microorganisms can limit excessive metal accumulation in plants through some mechanisms, therefore indirectly contributing to the growth and development of plants. Among the mechanisms used by microbes for the clean-up of heavy metals, biofilm formation along with EPS production seems to have better potential in the remediation efficiency of metal ions since biofilms with EPS offer various important advantages. Many previous studies have worked with planktonic microorganisms but we know that these free-living organisms are adversely affected by fluctuating environmental factors. As also stated by Nadeem et al. (2014), one of the main drawbacks in the inoculation of microbes is their inconsistent performance in fields. Biofilm formation may overcome these problems through the protective mechanisms of biofilm matrix. In addition, there are very few studies associated with biofilm-mediated remediation of metal-polluted soils. Microbial biofilms have high potential to be effectively used for the microbe-assisted phytoremediation of heavy metals from soils, as in the case of various environments, with respect to their planktonic counterparts; consequently, they can significantly increase plant growth and yield.

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CHAPTER 2

HEALTH BENEFITS AND PHARMACOLOGY OF BLACK SEED (*Nigella sativa* L.) IN THE MIRROR OF ITS HISTORY AND PHYTOCHEMISTRY

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1. INTRODUCTION

Nigella sativa L. (black cumin or black seeds) is one of the most frequently used traditional medicinal plants. It has been in used since ancient times. The scientific community, pharmaceutical firms, and health-conscious people are all showing increasing interest in black cumin. Esters of fatty acids, free sterols, steryl esters, lipase, phytosterols, and sitosterol are all present in the seeds of this plant. Thymoquinone (TQ) is one of the main ingredients of the essential oil. Antioxidant, antifungal, antibacterial, anticancer, and neuroprotective properties of TQ are well documented.

Some of major informations on Black Cumin is reviewed here according to ethnobotany, ethnopharmacology, phytochemistry and modern health approaches.

Basic nutritional needs are satisfied by plant-based foods, which also maintain the body healthy and provide defense against a variety of illnesses by boosting the immune system. Nutraceuticals and functional foods have gained popularity in recent decades. Research is ongoing to examine conventional foods with potential health benefits as the worldwide market for functional foods grows (Hannan et al., 2021). Plants have been utilized as herbal medicines for a very long time throughout Asia, Africa, and the Arab world (Shabnam et al., 2012). The use of medicinal plants as an alternative to conventional medicine in the treatment of ailments is currently receiving more and more attention. The potential of medicinal herbs in the treatment of diseases has been thoroughly investigated. Only 15% of the total of 300,000 medicinal herbs have been explored for their pharmacological properties (De Luca et al., 2012).

An annual herbaceous flowering plant known as "*Nigella sativa* L." with linear leaves, bluish flowers, fruits in the shape of capsules, and tiny black seeds that resemble cumin. It can reach a height of 20 to 30 cm (Hannan et al., 2021).

Figure. 1. *Nigella sativa* (whole plant, Flower and seeds) (Sharma et al., 2009).

2. BLACK CUMIN IN HISTORY

N. sativa is a member of the Ranunculaceae family and is conceivably one of the most important medicinal plants in human history. Various historical and theological text books make reference to it (Gilani et al., 2004). The plant's seeds are referred to as "Black cumin" in English, while they are known as "Habba Al-Sauda" or "Habba Al-Barakah" in Arab nations. This plant's common name in Urdu is "Kalonji." In Persian and Turkish, it is referred to as "Siyah Danch" and "Cork out," respectively. Love-in-a-mist, Habatul Barakah, Sonez, Krishana, Jiraka, and Sidadanah are further names for the plant (Sultan et al., 2012).

Ancient herbalists believed that black cumin, which is now known as a miracle herb or one of the Haba al-Barakah (blessed seeds) herbs, was the herb sent directly from heaven (Ahmad et al., 2013). Avicenna (Ibn Sina), a prominent physician of the 10th century and the founder of early modern medicine, highlighted various health-beneficial characteristics of black cumin in his well-known book "The Canon of Medicine," including improvement of the body's energy and recovery from fatigue and dejection (Yimer et al., 2019).

N. sativa is indigenous to South West Asia and Africa. Additionally, it is grown in Syria, Sri Lanka, Turkey, Pakistan, Bangladesh, India, and other Mediterranean countries. Egypt is known for producing high-quality seeds since Egypt has the best climate for cultivating this plant. *N. sativa* has a rich religious history and is cited in a number of religious writings. It is mentioned in the Bible's Old Testament, specifically in the Book of Isaiah, where it is referred to as "ketzah," a versatile spice for bread and cake (Naz, 2011).

Additionally, it is mentioned in both Indian and Chinese traditional medicine. It has been used for thousands of years to cure a variety of illnesses and is regarded as an essential substance in Indian medicine. The *N. sativa* has been discussed as "Habb-e-Sauda" in Traditional Arab and Islamic Medicine (Bukhari 5687; Ahmad et al., 2021).

Traditional medicine has recommended black cumin in the form of essential oil, paste, powder, and extract for a variety of illnesses and disorders, including hypertension, anorexia, amenorrhea, paralysis, rheumatism, headache, and dermatitis (Chaudhry et al., 2020).

Thymoquinone (TQ), thymohydroquinone, thymol, carvacrol, nigellidine, nigellicine, and α-hederin are primarily in charge of the pharmacological actions and therapeutic advantages of black cumin seed, especially its essential oil (Kooti et al., 2016). Numerous investigations into the chemical makeup of black cumin have revealed that its quinone component, also known as thymoquinone (TQ), is the primary factor contributing to its medicinal potential (Sahak et al., 2016).

3. PHYTOCHEMISTRY

It belongs to the Ranunculaceae genus of plants. Additionally known as the buttercup or crowfoot family. It includes about 2,000 species of flowering plants that are classified into 43 genera and are found all over the world. The Ranunculus genus is the largest. There are 600 species total, including *N. sativa* (Ahmad et al., 2021).

Black cumin's primary chemical family is the terpenes and terpenoids, which includes thymoquinone (TQ) and its derivatives. Black cumin contains quinine components, among which TQ is the most prominent. This presence is the primary cause of the variety in the pharmacological properties of black cumin. Several sterols are present in the oil obtained from black cumin, with -sitosterol accounting for the majority (44-54%). The second most important sterol in black cumin oil, stigmasterol, makes up between 16 and 16% of the total sterols (Cheikh-Rouhou et al., 2008).

p-cymene, carvacrol, thymohydroquinone (THQ), dihydrothymoquinone (DHTQ), -thujene, thymol, t-anethole, -pinene, -pinene, and -terpinene are some of the other substances included in plant (Sahak et al., 2016).

Figure 2. Black seed (*Nigella sativa* Linn.) flower (Ahmad et al., 2021)

4. HEALTH BENEFITS AND PHARMACOLOGY

Traditional medicine has used various *Nigella sativa* (NS) forms, including extract, oil, and powder, to treat conditions like fever, cough, diarrhea, bronchitis, and gastrointestinal disorders (Ramadan, 2007). TQ is the primary component of volatile oil and exhibits a wide range of pharmacological properties, including hepatoprotective (Hassanein et al., 2016), anti-inflammatory (Shaarani et al., 2017), antibacterial (Goel and Mishra, 2018), antioxidant (Erol et al., 2017), fungicidal (Almshawit and Macreadie, 2017), nephroprotective (K (Farkhondeh et al., 2017).

4.1. Anti-diabetic activity

Due to its ability to treat several ailments, cost-effectiveness, and lack of side effects when compared to synthetic medications, NS has a promising future in the prevention and treatment of diabetic disorders (Aisa et al., 2019). Due to their complementary effects, medicinal herbs have attracted the attention of many studies and are now viewed as an effective adjuvant to oral diabetes medications (Derosa et al., 2011). In addition, NS has been regarded as being less dangerous than oral diabetes medications (Ahmad et al., 2013).

Thymoquinone, one of the key bioactive molecules that was revealed to have a protective effect against diabetes, is primarily responsible for the therapeutic actions of NS (Khader & Eckl, 2014). Previous research showed that thymoquinone significantly raised the insulin levels in rats (Abdelrazek et al., 2018). It was also shown that the additional ingredients, including thymol, thymohydroquinone, dithymoquinone, nigellone, alpha-hederin, flavonoids, and fatty acids, contributed to the therapeutic effects of NS (Daryabeygi-Khotbehsara et al., 2017). The synergistic interaction between the many chemicals found in the plant extracts contributes to the effectiveness of NS medicinal effects. Furthermore, studies using both human and animal models showed that NS had no harmful side effects or toxicological impacts (Yimer et al., 2019).

After 12 weeks of therapy with 2 g/day NS as well as after 4 and 8 weeks of treatment with 2 g/day NS, Bamosa et al. (2010) demonstrated a significant decrease in FBG and 2hPG. According to Hosseini et al. (2013), the 2.5 mL NS oil group experienced a substantial drop in 2hPG levels and FBG when compared to the placebo group. After 12 weeks of treatment with 2 g/day and 3 g/day of NS, Bamosa et al. (2010) found a substantial reduction in HbA1c levels. After 12 weeks of treatment, NS at a dose of 2 g/day dramatically reduced the insulin resistance index and improved -cell function.

After 12 weeks of treatment with 2 g/day NS, Bamosa et al. (2010) demonstrated a substantial decrease in FBG, as well as in 2hPG after 4 and 8 weeks of treatment with 2 g/day NS. According to Hosseini et al. (2013), compared to the placebo group, the group receiving 2.5 mL NS oil experienced a substantial drop in 2hPG levels and FBG. Following a 12-week treatment with NS at doses of 2 and 3 grams per day, Bamosa et al. (2010) found a substantial drop in HbA1c levels. After 12 weeks of treatment, NS at a dose of 2 g/day dramatically improved -cell function and decreased insulin resistance index.

Figure 3. Fruit of *Nigella sativa* (Heba et al., 2009)

4.2. Antimicrobial, antiviral and antifungal activity

In healthy volunteers, "*N. sativa*" enhances T helper cells (T4), suppressor T cells (T8), and natural killer (NK) cell activity (Aljabre et al., 2015). Escherichia coli, Pseudomonas aeruginosa, and gram-negative bacteria Pseudomonas aeruginosa were examined for their concentration-dependent inhibitory effects on gram-positive bacteria "S. aureus" and diethyl-ether extract of "*N. sativa*" (Aljabre et al., 2015). The effects of the "*N. sativa*" extract were almost identical to those of the topical antibiotic mupirocin (Rafati et al., 2014). Black cumin showed excellent results against a variety of gram positive and gram negative bacteria that are resistant to many drugs, including resistant "S. aureus" and "P. aeruginosa" (Salman et al., 2008).

TQ of "*N. sativa*" is the primary cause of antifungal action. Dermatophytes, molds, and yeasts were resistant to the antifungal effects of TQ, thymohydroquinone, and thymol (Taha et al., 2010). Candida yeast growth is inhibited by the ether extract of "*N. sativa*" (Khan et al., 2003). According to reports, TQ suppresses Aspergillus niger and Fusarium solani activity in vitro in a way that is comparable to the antifungal medication amphotericin-B. (Al-Qurashi et al., 2007).

Its extract also shows some inhibitory effects on the human immune deficiency virus protease (Khan, 1999).

4.3. Anticancer activity

Numerous studies have demonstrated the powerful anticancer properties of black cumin and its components. A growing body of research indicates that the chemical components of black cumin seeds are chemopreventive and effective at slowing cell growth and inducing apoptosis. It has been claimed that giving black cumin seed ethanolic extract (250 mg/kg for 5 days) can reduce the development of liver cancer (Fathy & Nikaido, 2018). Thymoquinone from "*N. sativa*" has promise anti-mutagenic, anti-proliferative, anti-carcinogenic, and anti-neoplastic effects on a variety of tumor cells (Khan et al., 2011). In addition, it functions as a chemopreventive agent and is combined with therapeutic drugs to lessen the toxic side effects of treatment (Khader et al., 2007). Thymoquinone is the major constituent of *N. sativa* oil extract that induces apoptosis and inhibits proliferation in pancreatic ductal adenocarcinoma (PDA) cells (Chehl et al., 2009). TQ exhibits a down-regulatory effect on mucin 4 in pancreatic cancer cells (Torres et al., 2010). It was discovered that oral administration of TQ is effective in increasing glutathione transferase and quinine reductase actions and functions as a potential preventive source against toxicity in hepatic cancer and chemical carcinogenesis (Nagi and Almakki, 2009). TQ has been demonstrated to be helpful in the management of both hormone-sensitive and -refractory prostate cancer (Yi et al., 2008). TQ, the main bioactive of black cumin, was found to modulate many signaling systems in preventing the advancement of cancer because of its antioxidant potential (Mahmoud & Abdelrazek, 2019).

4.4. Anti-Inflammatory activity

Black cumin and TQ both have essential pharmacological characteristics that have anti-inflammatory effects (Dwita et al., 2019). According to Hossen et al. (2017), TQ suppressed pro-inflammatory factors on macrophage-like cells, such as nitric oxide (NO), nitric oxide synthase, and cyclooxygenase.

4.5. Gastroprotective activity

"*N. sativa*" oil raises mucin and glutathione levels in the stomach while lowering histamine levels in the mucosal gastric mucosa. As a result, it can be quite helpful in healing stomach ulcers brought on by ethanol and indomethacin (Rifat-uz-Zaman and Khan, 2004). TQ exerts a preventive effect against stomach ulcers by causing pepsinogen to activate pepsin in gastric juice (Kanter et al., 2006).

Figure 4. *N. sativa* (black cumin) (Heiss et al., 2011).

4.6. Neuroprotective activity

A number of neurological disorders, such as Alzheimer's disease, Parkinson's disease, ischemic stroke, acute brain damage, anxiety, depression, epilepsy, and schizophrenia have showed therapeutic promise when used in combination with black cumin and TQ. Additionally, in laboratory settings, black cumin and TQ were demonstrated to offer protection against a variety of chemical-induced neuronal damage. The antioxidative and anti-inflammatory activities of black cumin and TQ account for the majority of their neuroprotective potentials (Samarghandian et al., 2018).

TQ protects the organism from induced oxidative damage brought on by cisplatin-induced nephropathy (Badary et al., 1997). Black cumin boosts memory consolidation of stored information, inhibits loss of hippocampus pyramidal cells, promotes modulation of memory mutilation, and lowers neuronal cell death (Sayeed et al., 2013).

4.7. Antioxidant activity

Black cumin extract therapy reduced lipid peroxidation-related liver damage (Meral et al., 2001). One of the reasons people frequently like herbal medicines is their favorable safety profile. According to reports, combining black cumin with honey offers defense against oxidative stress and the development of cancer (Mabrouk et al., 2002).

Black cumin flavonoids enhance gastric mucus and strengthen mucosal immune defense by scavenging superoxide and hydroxyl free radicals (Badary et al., 2003). Inhibition of lipid peroxidation non-enzymatically has been revealed by TQ and black cumin oil (Houghton et al., 1995).

4.8. Anti-Obesity activity

The numerous preparations of black cumin have been investigated in order to discover new therapeutic agents from herbal remedies that fight obesity. Ahmed et al., (2017)'s research demonstrated that adding black cumin seed extract to the diet improved hyperlipidemic conditions by raising HDL levels and lowering LDL, triglyceride, and cholesterol levels.

4.9. Cardio protective activity

There have been many cardiac protecting properties of black cumin. Due to its antioxidant (Leong et al., 2013), diuretic (Zaoui et al., 1999), calcium channel blocking (Boskabady et al., 2005), and cardiac depressing qualities, black cumin reduces a number of risks associated with cardiovascular disorders (El Tahir et al., 1993).

CONCLUSIONS

According to reports, several black cumin extracts from various solvents have been proven to be effective antibacterial, antifungal, antiviral, and anti-parasitic agents. Since its discovery in 1995, TQ has been the subject of extensive research. When present in relatively high concentrations, "*N. sativa*" can both create ROS and act as an antioxidant when present in much lower amounts. Natural products like "*N. sativa*" have helped to the creation of new anticancer techniques and its primary ingredients such as TQ are intriguing prospects to remarkably resist cancer progression. It was characterized and documented how well they worked to stop various cancer-related processes like metastasis, angiogenesis, migration, and invasion. Also anti-diabetic, antimicrobial, antiviral and antifungal, anti-inflammatory, gastroprotective, neuroprotective, antioxidant, anti-obesity, cardio protective activities are well defined by numerous studies.

Most of the conducted investigations are limited to the pre-clinical level, further studies are required at the clinical level for a better translation of the obtained results on humans.

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CHAPTER 3

IMPLICATIONS OF CLIMATE CHANGE ON THE NASTY WEEDS OF FORAGES AND RANGELANDS

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INTRODUCTION

Agriculture is one of the sectors most impacted by climate change. Many people are impacted by a wide range of the effects of climate change in every culture. Around the world, climate change has an effect on a wide number of vital elements of life, including human health, agriculture, the ecosystem, food security, water supply, transit, and energy. The world's current livestock systems are seriously concerned about climate change. Animal health and production are impacted by climate variability and mean climate variable changes brought on by global warming, which also affects water and feed resources.

Climate change requires the processing, storing, transporting, selling, and consumption of livestock products. Consequently, it is debated whether the prevailing livestock systems can support livelihoods and supply the rising demand for animal products. Currently, the livestock industry is vital to the security and availability of food. With regional variations, livestock products (meat, milk, and eggs) account for around 30% and 6% of the production of ruminant meat and milk, respectively, on terrain that is typically unsuitable for agriculture, making up 15% and 31% of the world's per capita calorie and protein supply. Livestock also offers a broad variety of additional functions, including a source of draught power, a medium of transportation, a supply of nutrients for barren soils, a way to create revenue and diversify it, and a way to keep the money. These activities all contribute to the resilience and overall success of various communities. Around the globe, over 844 million people depend on agriculture for a component of their income, and the livestock business provides around 40% of the entire value of agriculture. The grazing systems of the globe. Despite the fact that the full breadth and degree of the implications are not yet known, climate change will affect how livestock contributes to food security and other sustainability problems.

The provision of ecosystem services has been impacted by ecosystem damage brought on by intensive land use and climate change (Scheiter et al., 02019). Climate change is defined by the Inter-governmental Panel on Climate Change (IPCC) as a shift in the climate's state that can be seen in variations in the mean or variability of its features over a long time—often decades or more. The causes of the variations in climate are either natural internal processes or external forcing (IPCC, 2014). Other significant contributors to climate change include livestock and people (Cheng et al., 2022). Agricultural industries also contribute significantly to atmospheric CO_2 emissions (Kuyah et al., 2021). Agriculture sectors are significantly impacted by weather and climate change (He et al., 2020; Lemi & Hailu, 2019; Pathak et al., 2018). The major effects of climate change on agriculture include rising temperatures, droughts, floods, desertification, and other extreme weather events.

According to research by Friedlingstein et al. (2021) and the IPCC (2021), the most recent decade's temperatures (2011-2020) are greater than those of the previous multi-century warm phase, which took place about 6500 years ago. Faster than in any prior 50-year period during at least the past 2000 years, the global temperature surface has grown since 1970. A magnificent "climate spiral" that depicts how monthly global surface temperatures have evolved since 1880 and the trend toward increasing global temperatures was recently revealed by NASA, which is similar to this.

Climate change's repercussions, which we have already started to perceive in recent years, will continue to reveal themselves (UNFCCC, 2021). Climate change influences animal growth rates, milk and egg production, reproduction rate, mortality and morbidity, and feed supply (Cheng et al., 2022; Torell & Lee, 2018). Rangelands' ecological services, such as cattle grazing, are similarly affected by increasing temperature and precipitation unpredictability (Torell & Lee, 2018; Khan et al., 2022a). According to Lee et al., rangelands are vulnerable to drought, desertification, and land degradation caused by both climatic and human activities (2021). According to Marambe and Wijesundara 2021 report, climate change, which includes extreme weather conditions such as heavy rainfall, floods, and droughts, impacts the prevalence and growth of poisonous weeds directly and indirectly.

According to Ziska and George (2004), increasing seed production and early seed maturity, prolonged seed dormancy, better seed dispersal, rich soil seed bank, higher seed germination rate, higher plant survival, and establishment ability of poisonous weeds than forage under a changing climate reduce rangeland ecosystems. According to several scientific assessments, climate change is a major danger to the world's rangelands. The threat of weed floras of forage and rangelands was examined in this chapter in relation to climate change.

Effects of Climate Change on Forage Production and Rangelands

The study conducted by the Global Carbon Project in 2021 indicates that compared to pre-industrial periods, the current impact of climate change on terrestrial and marine ecosystems is far worse (Figure 1). Rangelands are characterized by native plant communities and account for more than 40% of the Earth's terrestrial surface area. Grazing is the main land use in these areas (Angerer et al., 2016).

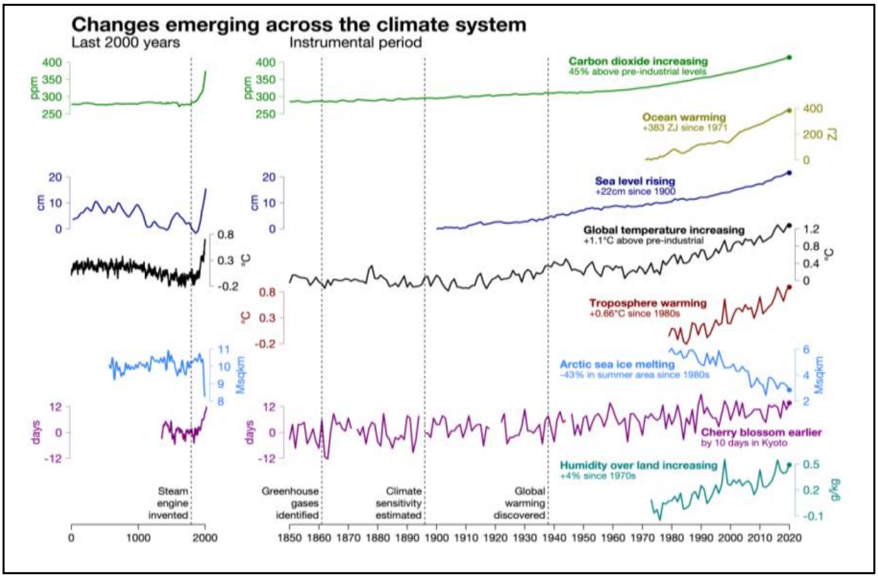

Figure 1. Impact of climate change on terrestrial and marine ecosystems

In addition to providing a variety of ecosystem services including food, fiber, water, recreation, and minerals, rangelands are essential to people's livelihoods all across the world, particularly in poor countries. Conflicting land uses, excessive grazing, extreme weather events, and socioeconomic changes are all contributing factors to the deterioration of rangelands around the world (Angerer et al., 2016; Chikowore et al., 2021; Dinan et al., 2021; Lee et al., 2021; Khan et al., 2022b). Rangeland degradation is characterized as a long-term loss in biological and economic productivity (Bedunah & Angerer, 2012).

Climate change has increased the spread and coverage of toxic plants in rangelands, causing rangeland degradation (Marambe & Wijesundara, 2021). According to Melo et al. (2022), climate change has a significant impact on forage production and quality in Azorean pastures. Most grassland areas of western and central Australia see a rise in summer and autumn rainfall, which has a substantial impact on the hydrology, vegetation, and stocking rate of the landscape McKeon et al (2009).

Climate change has resulted in an increase in the cover of perennial plants (Buzhdygan et al., 2016; Catlin et al., 2011; DiTomaso, 2000; Akshite et al., 2020; Uslu et al., 2022). According to McKeon et al. (2009), the quantity and quality of forage have decreased as a result of climate change's spatiotemporal change. Because of climate change, winter and spring annual grasses, which are greatly favored by cattle for their nutritional content, are being replaced by summer-growing permanent grasses.

According to Torell and Lee (2018) graze able forage production decreased significantly under the RCP 8.5 greenhouse gas scenario compared to current greenhouse gas concentrations (Figure 2). This illustrated how climate change affects both the quality and quantity of fodder production.

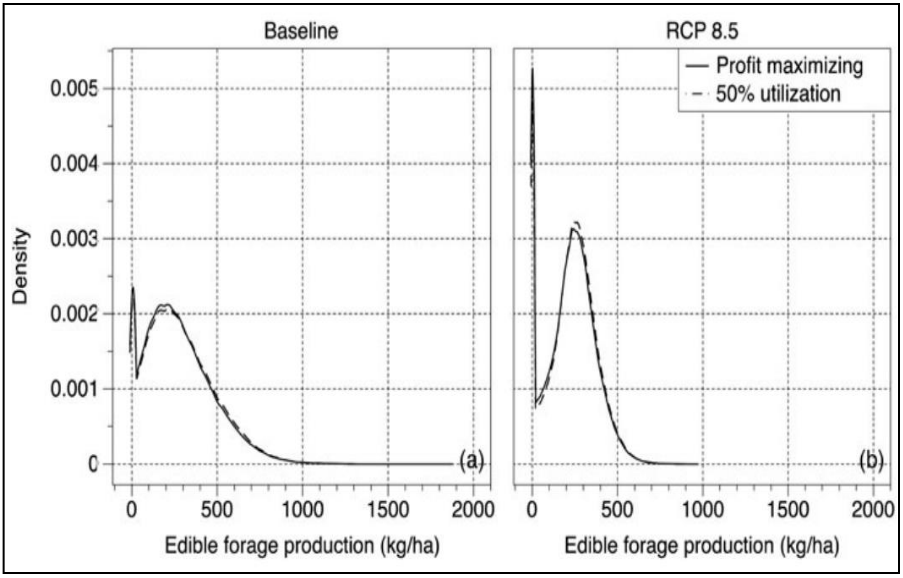

Figure 2. Kernel density estimates of (a) simulated graze-able forage production under the baseline greenhouse gas scenario; (b) graze-able forage production under the RCP 8.5 greenhouse gas scenario

POISONOUS WEEDS AND CLIMATE CHANGE

A poisonous plant is one that, when touched or ingested in big enough amounts, may be damaging or fatal to an organism, or any plant capable of triggering a toxic and/or lethal reaction (Hall et al., 2020; Gupta, 2018). The economic impact of hazardous plants of livestock can both direct and indirect losses (Godde et al., 2021). Direct losses are effects on animals such as death, abortions, birth defects, decreased fertility and decreased organ function. Indirect losses are management costs and include increased medical treatments, decreased land values, lost time to increased management, and increased feed requirements (Hall et al., 2020). Most dangerous plants have established root systems and are more competitive than forage in the water, soil, and other supporting resources (Zhao et al., 2010).

According to Torell & Lee (2018), broom snakeweed is only one of the numerous possible dangers to rangeland ecosystems brought on by climate change. Many researchers discussed that overgrazing (Catlin et al., 2011; Chikowore et al., 2021; Stegelmeier, 202) and climate change (Akshit et al., 2020; Dinan et al., 2021; Godde et al., 2021; Scheiter et al., 2019; Ziska & Dukes, 2011) is resulted decreased the forage grasses and increased the expansion of poisonous weeds, finally, the grassland is degraded gradually. Alkaloids, glycosides, organic acids, resins, phytotoxins, minerals, mycotoxins, and miscellaneous unknown poisons are the most common types of poisons (Binder et al., 2010; Buzhdygan et al., 2016; Cortinovis & Caloni, 2015; Gupta, 2018).

TOXIC SUBSTANCES ARISE FROM POISONOUS PLANTS

I. Alkaloids

Nitrogenous chemicals known as alkaloids are generated naturally by plants and have a bitter taste and complex structure (Buzhdygan et al., 2016; Cortinovis & Caloni, 2015; Ozkan, 2015). Phalaris aquatica, often known as "Phalaris stragger," causes a disease known as *Phalaris arundinacea* paralysis (Binder et al., 2010; Ozkan, 2015). When *Phalaris arundinacea* is fed to animals, they develop rough hair coats and excessive eye watering in addition to having less milk produced and losing weight. Leguminosae plants like Lupinus and Lathyrus contain very poisonous alkaloids (Binder et al., 2010). The distribution and quantity of rainfall as well as the rising temperature affect the alkaloids' concentration (Scheiter et al., 2019).

II. Glycosides

Plants produce very poisonous glycosides (Hall et al., 2020). Among these, glucosinolates, cyanogenic glycosides, coumarins, and saponin are the most prevalent. According to Ozkan (2015), physiological variables that impact glycoside concentration include nutrition and water deficit, development stage, accumulation in specific plant tissues, geographic or topographic location, and seasonal effects of soil and climate.

III. Hydrocyanic acids

The most typical cause of Jonson grass is hydrocyanic acid (HCN), also known as prussic acid (Hall et al., 2020). When under stress, such as drought, wilting, frosting, or stunting, the glycoside breaks down to generate free HCN, which is particularly poisonous to cattle (Strickland et al., 2017).

IV. Mycotoxins

Certain species of mold naturally create mycotoxins, which are deadly chemicals (fungi). The amount of mycotoxin contamination is influenced by the geographical location and climate (khan et al., 2022b; Ozkan, 2015).

IMPORTANT POISONOUS WEEDS OF FORAGES AND RANGELANDS

1) **Copperweed (***Oxytenia acerosa* Max Licher**)**

High salt content and enough moisture are ideal for copperweed growth. This perennial weed has a high alkaloid level and is poisonous to all cattle. This weed is more poisonous at the mature leaves stage (Hall et al., 2020).

Photo 1. Copperweed (Oxytenia acerosa) Max Licher

2) **Loco weeds** (*Astragalus spp.)*

Alkaloids found in locoweeds (*Astragalus* and *Oxytropis* genera) are toxic and result in the classic "Loco" poisoning. There are roughly 1,000 kinds of herbaceous plants that make up astragalus (Nelson et al., 2007). The entire locoweed plant is poisonous. Young animals suffer the most because maturing neurons are more sensitive to the toxin's effects. Despite growing in a variety of plant groups and on a variety of soils, locoweed frequently occurs in arid, open areas. The plant is poisonous in all development stages including after drying (Forero et al., 2011).

Photo 2. Loco weed (*Astragalus spp.)*

3) Desert tobacco (*Nicotiana obtusifolia* Max Licher)

A member of the Solanaceae family is desert tobacco (Nelson et al., 2007). The agricultural weed desert tobacco plant is harmful and repulsive to cattle (Gupta, 2018). Acute Deseret tobacco poisoning is characterized by shaking, twitching, staggering, paralysis, convulsions, heavy breathing, coma, and death (Forero et al., 2011).

Photo 3. Desert Tobacco (*Nicotiana obtusifolia*) Max Licher

4) Johnson grass (*Sorghum halepense L*.)

Johnson grass is a warm-season grass. It is adapted to a wide range of soil types and climatic conditions (Rocateli & Manuchehri, 2019). The most common cause of Jonson grass is hydrocyanic acid (HCN), also called prussic acid. Weakened animals that foam at the mouth, blood is bright red finally abortion and death are the symptom and causes of Johnson grass (Hall et al., 2020).

Photo 4. Johnson grass (*Sorghum halepense L*.)

5) Cocklebur (*Xanthium spp.)*

Cocklebur (*Xanthium spinosum* and X. *strumarium*), an invasive native annual plant, are triangular or heart-shaped and covered in extremely fine hairs on both sides. The burs are coated in stiff, hooked spines and are dark or greenish brown in hue. They contain two seeds. In the state's moist waste regions, cockleburs are usually found at lower altitudes (Forero et al., 2011). Old fields, overgrazed pastures, floodplains, and stock watering areas are examples of typical ecosystems. The main culprit behind Cocklebur weeds is glycosides (Gildersleeve et al., 2013).

Photo 5. Cocklebur (*Xinthium strumarium*) weed

6) Poison Hemlock (*Conium maculatum* L.)

The whole plant is hazardous, but the root and seeds in particular are very lethal (Gildersleeve et al., 2013; Nelson et al., 2007). Poison hemlock is distinguished by its hollow stems and small, umbrella-shaped white flowers. It poisons many kinds of animals as well as humans. Low-waste regions with rich, dry, or wet ground are where you may find it (but not saturated). The alkaloid in these plants is often the most dangerous (Gildersleeve et al., 2013).

Photo 6. Poison Hemlock weed

7) Jimson weed (*Datura spp.*)

The solanaceae family includes this weed (Nelson et al., 2007). It has hollow, erect, branching stems that are annual in nature. Although the nectar and the whole plant are deadly, it is the seeds that often result in poisoning. Usually, scopolamine, atropine, and other anticholinergic alkaloids are to a fault (Gildersleeve et al., 2013)

Photo 7. Flower, Pod and Leaves of Datura Spp.

8) Blood root (*Sanguinaria canadensis* L.)

It belongs to the papaveraceae family and the most common name is blood root and red Puccoon (Nelson et al., 2007). All parts of the plant contain alkaloids and more concentrated in the root (Nelson et al., 2007).

Photo 8. Blood root weed

9) Night shades (*Solanum spp.*)

Solanum is a very large genus with 1,700 species (Nelson et al., 2007). Nightshade species' toxicity can vary widely depending on the environment, the parts of the plant ingested, and the degree of plant maturity (Forero et al., 2011). Solanine and other glycoalkaloids have predominantly gastrointestinal irritant effects (Gildersleeve et al., 2013; Nelson et al., 2007)

Photo 9. Nightshades

10) Snakeweed (*Gutierrezia sarothrae*)

A species of flowering plant belonging to the Asteraceae family called *Gutierrezia sarothrae* is sometimes referred to as broom snakeweed, broom weed, snakeweed, and match weed. Snakeweeds are facultative selenium absorbers, meaning they can grow in soils without selenium but will still accumulate selenium (Hall et al., 2020). It is an invasive plant that carries chemicals that can kill animals and induce abortions in them.

Photo 10. Snake weed

11) Lady of the night (*Brunfelsia lactea*)

Brunfelsia lactea belongs Solanaceae family. Dogs are very poisonous to every species of Brunfelsia, despite the fact that they often eat its berries. Although the exact toxic components are unknown (maybe alkaloids), Brunfelsia toxicity causes severe gastrointestinal distress that quickly progresses to generalized seizures (which may or may not be treated with medication), breathing problems, and cardiac dysrhythmia, followed by cardiac arrest and death. No antidote exists, the only available therapy is symptomatic, and life is not assured (mortality appears to be very high).

Photo 11. Lady of the night

12) Kudu lily (*Pachypodium saundersii*)

Pachypodium saundersii belongs to family Apocynaceae. Because cardenolide (cardiac) glycosides are present, all Pachypodium species are toxic. *P. saundersii* is one of the most dangerous plants, and African tribes people utilize it to create poison arrows that may kill large game animals.

Photo.12 Kudu lily

13) Parthenium Weed (*Parthenium hysterophorus*)

The alien invasive *Parthenium hysterophorus* L. A member of the Asteraceae family, also known by the names white top, whitehead, congress grass, and carrot grass. It is a 2 m tall, upright annual plant with alternate, deeply divided leaves. Given that this is one of the most dangerous toxic plants known to humans and animals, it is both very beautiful and rather frightening. To those who are vulnerable, every component of the plant may produce severe dermatitis. Asthma and other chronic respiratory diseases may be brought on by pollen and dried plant hairs that are dispersed in the air. Given enough concentration, this plant is the only one I'm aware of that may poison people via the air (apart from pollen allergies).

Photo. 13 Parthenium Weed

14) *Heliotropium haussknechtii* Bunge

While *Heliotropium haussknechtii* plant is poisonous in all sections, people may only get poisoned from little amounts of it. It may cause liver failure in horses, however, and is hazardous to them. Animals that have no other options for food or when hay is infected will still consume it, despite its unpleasant taste.

Photo. 14 *Heliotropium haussknechtii*

15) Redroot pigweed (*Amaranthus retroflexus L*)

Amaranthus retroflexus may accumulate nitrates and contain soluble oxalate. If large quantities of immature pigweed stems high in nitrates are consumed, ruminants are at risk of nitrate poisoning. Additionally, redroot pigweed contains an unidentified toxin that results in renal tubular nephrosis.

Photo. 15 *Amaranthus retroflexus* L

CONCLUSION

The prospective repercussions of climate change on contemporary livestock systems across the globe are a substantial issue, even though the subject gets less focus in international assessments like those released by the IPCC. There is considerable evidence that consequences will occur throughout the supply chain, from farm production to processing operations, storage, transportation, retailing, and human consumption, even though estimating the total implications of climate change on the cattle sector is beyond our present knowledge. While climate-related risks vary greatly dependent on the environment, they are expected to be more severe in already hot locations where there are insufficient institutional and economic resources for adaptation. There are enormous uncertainty regarding climate futures, as well as the exposure and reactions of interrelated human and ecological systems to climatic changes across time. As a result, adaptation choices must account for a broad variety of alternative futures, including those with low likelihood but substantial effects. Climate change which indicates decrease in rainfall and rise in temperature events influences the quantity and quality of fodder and diminishes the rangelands. Changing climate boosted the abundance and coverage of noxious plants in rangelands that cause range land degradation. The distinguishing properties of deadly weeds, which are more competitive than forages in water, soil, and other supporting resources, include capacity to adapt to challenging situations by growing root systems and tolerance to changeable weather settings. Observe pastures, identify any damaging plants, and learn about the biology and ecology of such weeds.

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CHAPTER 4

MANAGEMENT AND UTILIZATION OF WATER HYACINTH (*Eichhornia crassipes* Mart.)

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INTRODUCTION

The biggest problem in the 21st century is the lack of fresh water and the contamination of existing supplies. Therefore, it is crucial to save water for future generations to manage the water crisis, ensure the sustainable use of water for drinking, household, industrial, and agricultural purposes, and to preserve a healthy environment. One of the main contributors to future water shortages and pollution is aquatic weeds, which are the main offenders in degrading fresh water and impeding their intended usage in a variety of ways. Along with lovely and scenic rivers, dams, streams, etc., Pakistan has one of the greatest canal systems in the world. Aquatic weed infestations are prevalent in most of these water bodies, and they are susceptible to spreading rapidly if effective management measures are not put in place on the plains, where water flow is sluggish and conducive to aquatic weed infestation.

Temperature rises are anticipated to disturb current patterns of animal and plant dispersal in aquatic habitats. Changes in rainfall and runoff influence the amount and quality of aquatic organisms' habitat, which indirectly affects the efficiency and diversity of the environment (Khan et al., 2022a; Khan et al., 2022b; Uslu et al., 2022). Streams, rivers, lakes, ponds, irrigation canals, barrages, drainage ditches, dams, and rice fields are just a few examples of freshwater that are frequently infested with aquatic weeds. These weeds reduce crop yield, obstruct water flow, are unsightly, and interfere with inland water recreation. Native aquatic plants, commonly known as exotic or alien species, are an inherent part of freshwater ecosystems and are crucial for the preservation of robust aquatic ecosystems, the prevention of shoreline erosion, and the removal of pollutants from water. Native aquatic plants are a naturally occurring element of fresh water that can outgrow to totally take over natural habitats, as opposed to invasive plants, often known as exotic or alien species, which can become severe weed issues and must be managed rigorously. By developing several methods of reproduction and adjusting to various environmental factors, invasive weeds grow quickly and colonize enormous regions. They significantly damage the environment and the economy. Aquatic weeds are also said to decrease crop output in agricultural production regions, obstruct water flow in irrigation canals and waterways, create ugly sceneries, and interfere with leisure activities in inland waters (Ramlan, 1991). The various applications and management of water hyacinth are categorized and described in this chapter.

Botanical Description of Water Hyacinth

Water hyacinth (*Eichhornia crassipes* Mart.) is a floating aquatic plant with infrequently established mud roots and inflated petioles (Hutchinson and Dalziel 1968). Water hyacinth is recognized by rosettes of round, waxy, bright green leaves connected to wide, spongy (sometimes bulbous or inflated for buoyancy) petioles, black fluffy roots that often float underneath the floating plant, and gorgeous purple flowers when in bloom. The odd inflorescence includes an above-ground spike that may grow as tall as 30 cm. The fruit is a three-chambered seed capsule with six stamens on the blossoms (Langeland and Burks 1998).

Photo 1: View of water hyacinth plant

Photo 2: View of water hyacinth flower

Adaptation of Water Hyacinth

In most of the world's frost-free zones, water hyacinth is a common freshwater weed and is regarded as the most problematic aquatic plant (Holm et al., 1997). Low temperatures are intolerable, but it can live above 12 ℃. Its maximum growing temperature is 33 ℃, with 25 to 30 ℃ being ideal for its lush development (Kasselmann, 1995). Water hyacinth has some freeze resistance even if it cannot grow in cold locations owing to temperature requirements. The somewhat frozen regions of the weed's above-water segments may fast regenerate from submerged stem ends that are shielded from freezing by water (Langeland and Burks 1998).

Origin, Distribution and Potential Spread

German scientist C. von Martius made the discovery of the water hyacinth in 1823 while researching the Brazilian flora. *Pontederia crassipes* was its given name. As Kuntz had described it in 1829, Solms put it in the genus *Eichhornia* after sixty years, and now it is extensively distributed across the nations of Central and South America after being imported as an ornamental in the 1890s (Prefound and Earle 1948). According to Lei and Bo (2004), the water hyacinth has spread to 62 nations in all of Asia, Africa, Oceania, and North America. According to Fawad et al. (2013), water hyacinth is a common plant in practically all water bodies, particularly the streams in Swabi District, Pakistan.

According to (Lei and Bo 2004), water hyacinth originated in the Brazilian state of Amazonas and is currently spread by people to other South American states in the tropics and subtropics. The present and global distribution of water hyacinth was documented by Julien et al. in 1999. According to Rezene (2005) water hyacinth is found in both tropical and temperate regions of the globe. Currently, water hyacinth may be found in the tropics and subtropics between 39°N and 39°S. In spite of being extensively distributed in Australia, North America, and Africa, water hyacinth is well known in China (Jianbo et al., 2007). According to Motwani et al. (2013), water hyacinth is an alien weed that has become invasive in India.

Invasion of Water Hyacinth in Pakistan

Several invasive aquatic weeds are widespread in Pakistan, including *Salvinia molesta, Hydrilla verticillata, Pistia stratiotes, Lemna, Typha*, and *Eichhornia crassipes*. Among these, water hyacinth is often regarded as the most troublesome aquatic weed in Pakistan because of the habitats it destroys for fish and other species by creating a thick, impenetrable mat over the water's surface. Water hyacinth has reportedly overrun water streams, lakes, and stagnant water bodies in the Swabi District, Khyber Pakhtunkhwa, Pakistan (Fawad et al., 2013). Water hyacinth aquatic weed is creating severe environment-related problems in Khyber Pakhtunkhwa province and due to this reason, its management is very important to avoid the further spread of this invasive weed.

Photo 3: View of water hyacinth infestation

Fast Vegetative Growth of Water Hyacinth

Up to 12 % more biomass may be produced each day by water hyacinth. It has been estimated that it takes between 6 and 15 days for biomass or population to double (Gopal, 1987). The foliage is quite thick, as would be predicted, with values for the leaf area index of 7.8 and 5.8 found in research in Florida, which are similar to many of the most productive terrestrial ecosystems (Knipling et al., 1970). According to (Kateregga and Sterner 2007), water hyacinth has a very quick growth rate and entered Lake Victoria in 1992-1993. It then began to develop rapidly and reached its maximum level in 1997–1998.

Problems and Interference with Human Resources

Water hyacinth is known as the most problematic aquatic weed in the world (Stroud, 1991). According to Rezene (2005) water hyacinth alters native aquatic plant and animal ecosystems, generates significant economic losses, and interferes with a number of water-related functions, including irrigation, the production of power, fishing, and navigation. Due to its opulent growth, it has the capacity to quickly take over large streams.

It blocks water channels, rivers, and streams, reduces sunlight penetration, destroys natural wetlands, lessens the aesthetic value of waterways, eradicates native aquatic plants, alters the pH and oxygen levels of water, lessens gas exchange at the water's surface, raises the temperature, causes significant water loss through transpiration, and restricts recreational water use. Insects are protected by water hyacinth, which also spreads illnesses that are harmful to people's health (Jianbo et al., 1999)

Threat to Biodiversity and Ecosystem

By obstructing the air-water interface and lowering oxygen levels, water hyacinth mats degrade the quality of the water, which has a negative impact on aquatic life, including fish and other creatures. The water hyacinth significantly reduces biological diversity, eliminates nearby submerged plants by obstructing sunlight, changes emergent plant communities by driving them away and destroying them, changes animal communities by obstructing access to the water, and furthermore eradicates nearby plants that animals rely on for food and shelter. These effects were discussed by Gowanloch, 1944. According to Masifwa et al., 2001 research, water hyacinth has grown very quickly on Uganda's Lake Victoria, posing a danger to the environment and the variety of marine life.

According to Li et al. (2004), water hyacinth is an alien invasive plant that threatens the ecosystems and natural species of Shanghai's Huangpujiang watershed. According to Jianbo et al. (2007), water hyacinth invasion has contaminated a number of water streams, rivers, and lakes over about half of China's landmass, causing a decline in flora and fauna diversity, a serious danger to biodiversity, and a variety of ecosystem services. Multiple concerns, ranging from social and economic to ecological, are associated with water hyacinth and they often endanger biodiversity (Patel, 2012). As per Adebayo et al. (2011), global warming is a result of water in tropical and subtropical regions endangering biodiversity and changing ecological conditions.

Economic Losses

Chu et al. (2006), discussed that at the start of the 21st century, the introduction of alien invasive plant species, especially the water hyacinth, caused major environmental issues and ecological risks in China, as well as a significant financial burden of almost $7 billion USD annually. According to Fayad et al. (2001), water hyacinth infestations in Egypt result in an estimated 3.5 Billion L of water being lost yearly, which would be enough to irrigate 432 km^2 of land. Water hyacinth growth on Victoria caused significant economic losses, according to Masifwa et al., 2001 research. In the area between 40°N and 45°S, water hyacinth is said to generate significant social and economic losses, according to Lei and Bo's 2004 assessment

Water hyacinth causes significant economic losses and obstructs the usage of water (Julien et al, 1999; Charudattan, 2001; Rezene, 2005). Water hyacinth is an invasive plant that causes significant economic losses, according to Li et al. (2004). According to Jianbo et al. (2007), water hyacinth has a significant negative impact on the global economy.

Threat to Native Fish Communities and other Micro-Fauna

Given that many bodies of water serve as fish traps, water hyacinth may have an influence on fish migration. Fish colonies and other aquatic micro-fauna are impacted when water recedes because they are unable to escape during floods while swimming over with water hyacinth to the stream banks. According to Masifwa et al. (2001), water hyacinth poses a serious danger to the variety of marine species on Lake "Victoria" in Uganda. It has been observed to significantly increase evapo-transpirational losses as well as fish losses. Due to its massive mats that it produces on the water's surface, water hyacinths restrict oxygen levels, which in turn affects fish and other aquatic life. They may also lead to specie shifting (Lei and Bo 2004). Water hyacinth have dramatic impacts on the fish population in Lake Victoria, according to (Kateregga and Sterner 2007).

Photo 4: Sever infestation of water hyacinth in water body

Block Water Ways

In Egypt, aquatic bodies are severely harmed by water hyacinths (Fayad et al., 2001). Water hyacinth mats that are thickly woven around rivers and streams obstruct waterways and impair the effectiveness of hydropower energy production (Lei and Bo 2004). Jianbo et al. (2007) observed that a water hyacinth infestation in China's territory hampered water flow, causing damage to irrigation infrastructure, navigational issues, and hydroelectric power production. Water hyacinth, according to Malik's 2007 assessment, seriously impairs irrigation systems and obstructs waterways, which affects navigation and hydropower production. Water hyacinth, according to Patel (2012), prevents shipping, farming, and recreational activities in freshwater areas.

Photo 5: View of waterways blockage due to water hyacinth

UTILIZATION OF WATER HYACINTH

Phytoremediation and Waste Water Treatment

Through its ability to absorb several sorts of contaminants, including heavy metals found in water, hyacinth plays a significant part in the reduction of pollution. Minerals and inorganic compounds from sewage can be absorbed by hyacinth. It may grow quickly in a variety of settings. As a result, it is brought in for use as an organic fertilizer, such as compost or mulching, after being used to treat sewage. Hyacinth may take up dissolved pollutants, nitrogen, phosphorus, algae, suspended particles, and other nutrients. It has been applied to sophisticated water treatment, sewage treatment, and hospital wastewater treatment.

Water hyacinths may be used as a biological filter system for waste water treatment. Water hyacinth may be utilized for large-scale waste water treatment since accumulation of heavy metals including Zn, Fe, Cr, Cd, and Cu has no deleterious effects on it (Mishra and colleagues, 2008). Water hyacinths purify water by removing a number of heavy metals and other contaminants (Pinto et al., 1987; Delgado et al., 1993; Zaranyika et al., 1994; Mahamadi and Nharingo, 2007, 2010a, 2010b). Water hyacinth is employed for phytoremediation and wastewater treatment, according to Malik (2007). Water hyacinth is the best wastewater treatment solution because some organic compounds in water are carcinogenic at concentrations 10,000 times greater than in surrounding water. Water hyacinth roots absorb pollutants such as Pb, Mg, and strontium-90 from sewage.

Ornamental Purpose

The water hyacinth was initially used as an ornamental crop in a range of countries more than a century ago due to its enticing appearance and aesthetic value in the environment. Many nations use water hyacinth for decoration because it has lovely bulbous leaves and purple flowers. According to Adebayo et al. (2011), water hyacinth is often marketed in southern Ontario.

Bio-Energy Source

They were seen as a substitute for fossil fuels in the twenty-first century since numerous studies discovered they could transform their content into fuel energy for less money and were acknowledged as an environmentally beneficial product. Water hyacinth biomass has the potential to displace conventional fossil fuels and maybe develop into a renewable resource over the next ten years since it is one of the species with the fastest rate of development. This goal is essential to halt the depletion of fuel supplies and satisfy the growing worldwide demand for energy. As an alternative to fuel energy, the dried biomass may also be formed into briquettes, which are useful as a co-firing agents in coal power plants. Therefore, in the future, the production of briquettes from compacted biomass leftovers may reduce the need for coal to provide more energy.

Due to its rapid pace of growth, water hyacinth makes an excellent source for biogas production. According to Malik (2007), water hyacinth is a great source for producing methane. The experiment conducted in Washington, DC in 1976 revealed that a water hyacinth mature crop generates greater than 70,000 m^3 of biogas ha^{-1}. While in contrast to pure methane (895 Btu/ft^3), one kilogram of dry materials may generate 370 L of biogas with a calorific value of 22,000 kJ/m^3 (580 Btu/ft) (Washington DC. 1976).

Bengali farmers collected and piled these plants for drying at the beginning of the winter season (Reddy and Tucker, 1983). Once that was accomplished, they used the dried water hyacinths as fuel and the ashes as fertilizer, producing an experimental maximum of more than half a ton per day. About 50 L of ethanol and 200 kg of leftover fiber are produced from a ton of dried water hyacinths in India (7,700 Btu).

Bacterial fermentation of biomass yields 26,500 cu ft (600 Btu) of gas, which is comprised of 51.6% CH_4, 25.4% H, 22.1% CO_2, and 1.2% O. One ton of dry mass may be synthesis gas by air and steam at high temperatures (800 °C) to create about 40,000 ft^3 (or 1,100 m^3) of natural gas (143 Btu/cu ft), which comprises 16.6% H3, 4.8% CH_4, 21.7% CO, 4.1% CO_2, and 52.8% N. The high levels of moisture in water hyacinths often impede corporate operations and sharply increase handling costs. To optimize capital inputs, a continuous, hydraulic production system may be created in place of traditional agriculture, which is mostly a batch process (CSIR, 1948-1976).

As per Benemann (1981), putting collection stations and processors on ponds that benefit from prevailing winds may reduce the labor-intensive job is to collect water hyacinth. Systems for treating wastewater might be added as a bonus for this company. The biomass would next be transformed into ethanol, gas nitrogen, fertilizer, and/or hydrogen. The produced water byproducts and fertilizer may be used to irrigate neighboring farmland (Duke, 1981).

A an Organic Garment Fiber

Textile scientists are always searching for fresh sources of fiber and more efficient ways to handle materials sustainably. They are working on to develop a fiber that is completely natural, ideally organic, and may be utilized in the textile industry for upholstery, interior design, and garments. Although hyacinth fabric is not currently offered in your neighborhood markets or shops, keep a watch out for this ground-breaking development in eco-friendly clothing design.

As a Food

In Taiwan, local people consume the plant as a carotene-rich table vegetable. Javanese sometimes prepare and consume the flower and green components (Duke, 1981). In fact, fried hyacinth beans help improve digestion. Eichhornia beans are used in traditional Chinese medicine to maintain a healthy spleen. The plant is also used to cure worms, diarrhea, upset stomach, and other conditions.

Makes Skin Healthy

Hyacinth is an ingredient in several skincare treatments. Water hyacinth is an excellent option for treating a variety of skin-related issues due to its antimicrobial, antifungal, and antibacterial qualities. The horse skin may be treated with flowers in Kedah (Java). They can be used as an animal tonic (Wain, 1981; Oudhia)

Powerful Anti-Inflammatory

Hyacinth is popularly used as an anti-inflammatory by people in the Philippines. It is one of the herbal plants that may be found in the Philippines. They prepare hyacinth juice, combine it with vitamin-rich lemon juice, and then apply it topically to a boil. It thus lowers abscess and soothes inflammation.

Especially For Women

This herb can genuinely help women build a healthy body. This water hyacinth plant is used by some Kenyan women to encourage breastfeeding. To maximize its benefits, cooked hyacinth can be consumed by new mothers. However, its blooms can benefit ladies who have irregular periods.

GENERAL USES

The water hyacinth is a soil supplement for sandy soil, which may enhance the soil's hydro-physical and chemical properties and provide growing crops with a variety of nutrients. Water hyacinths from Lake Victoria are used to build rope, furniture, and wallets in East Africa. Even while researchers (Nolad and Kirmse 1974) reported that water hyacinths were only sometimes utilized to make paper, (Aguilo et al., 2007) claimed that this was the case. This is true even though the fertilizer's high alkaline pH value, which is also utilized as organic fertilizer and animal feed, is the subject of debate.

Water hyacinth's abundant biomass can be used for a variety of purposes, including the removal of heavy metals, the remediation of dyes, the treatment of wastewater, the production of bioethanol and biogas, industrial uses, the production of electricity, the production of medicines, food and antioxidants, agriculture, and sustainable development (Patel, 2012)

MANAGEMENT OF WATER HYACINTH

Physical/Mechanical Control

Water hyacinth is a significant issue in Pakistan's drainage ditches and streams. Water hyacinths complete their whole life cycle in the water bodies, adding nutrients as a result. Because of the availability of nutrients, accelerates the issue. In Pakistan, this weed has not yet been used, particularly in rural regions, and it poses a danger to the aquatic ecology. For this reason, mechanical management is the most environmentally benign option with no signs of water pollution. On a modest scale, hand pulling may be used to physically control water hyacinth. Because of how difficult it is, it should only be utilized in situations when the rate of removal can outpace the rate of regrowth. Deep water removal is possible with the use of certain nets and obstacles.

However, mechanically, the enormous mats are chopped into pieces and, depending on the size of the water body, may be manually or mechanically carried out to the stream bank using an excavator, a tractor, or boats. On a wide scale, combine harvesters are appropriate for controlling water hyacinth, which cuts, collects, and brings biomass to the stream banks, according to Wade, 1990. Wade indicated that the employment of heavy machinery is essential in case of high infestation. The finest shredding device for mechanically controlling water hyacinth, according to Tung (2004), is Aqua Terminator. Due to the water hyacinth's rapid vegetative growth and potential for re-establishment, mechanical control alone cannot guarantee the complete biomass suppression of the plant. Instead, a long-term control program that establishes monthly inspection of water bodies and cost-effective yet effective control measures is required.

Photo 6: View of physical /mechanical control of water hyacinth

Biological Control

According to Harley (1990), the moth *Sameodes albiguttalis* and two weevils called *Neochetina eichhorniae* and *Neochetina bruchi* are the best pests for controlling water hyacinth. Labrada talked about the problems with the water hyacinth in 1996 in relation to many countries in Latin America and Africa. According to him, *Sameodes albiguttalis, Cercospora rodmanii*, and *Alternaria eichhorniae* are some potentially promising biological control agents as well as the most common weevil species, *Neochetina* spp., for controlling water hyacinth.

According to Julien et al. 1999, two bio-control agents that are used internationally to eradicate water hyacinth are *Neochetina bruchi* and *Neochetina eichhorniae.* Weidong et al. 1999 cited that *Neochetina eichhorniae* for the control of water hyacinth. Mallya et al. (2001) described utilizing two weevils in Tanzania for bio-control water hyacinth: *Neochetina bruchi* and *Neochetina eichhorniae*. Six insect species, comprising two weevil species (*Neochetina eichhorniae)* and (*Neochetina bruchi*), two moth species (*Xubida infusellus*) and (*Niphograpta albiguttalis*), a bug species (*Eccritotarsus catarinensis*), and a mite species, were used to control water hyacinth in Africa (Julien, 2001). For the management of water hyacinth in Egypt, *Neochetina bruchi* and *Neochetina eichhorniae* have been discovered as two biocontrol agents (Fayad et al., 2001). (Kathiresan, 2000) explained how insects and fungus infections were used in India's traditional bio-control of water hyacinth.

Allelopathic Potential of Certain Plants against Water Hyacinth

As a bio-agent for the management of water hyacinth, the allelopathic capability of several plants was assessed. According to Saxena 2000, *Lantana camara* L. is an allelopathic plant that may inhibit the formation of water hyacinth. Water hyacinth was said to be destroyed by 10 gl of Omavalli (*Coleusam boinicus* L.) powder, according to Kathiresan in 2000. *Lantana camara* L. extracts are extremely poisonous and inhibit the development of water hyacinth, according to (Zheng et al., 2006; Motwani et al., 2013) reports. According to (Kong et al., 2006), the lantana plant contains three allelochemicals: phenolic acid, lantadene A, and lantadene B. However, only lantadene A and lantadene B are effective against the control water hyacinth.

Solarization

Water hyacinth can be controlled via solarization, according to Ogari and Knaap (2002), who carried out an experiment on Victoria Lake to assess the effectiveness of this method. They used polythene sheets and mats made of water hyacinth as well as covering the plastic edge approximately one meter below the surface. They divided the aggressive plants into six equal groups and covered them with polythene sheets in three different gauges, two different colors (black and absolutely transparent), and six distinct groups (gauges 500, 750, and 1000.). Every polythene sheet had a small hole punched out for a thermometer to collect temperature information. After the information was captured, the hole was sealed with cello tape. At 6:00 am, 8:00 am, 12:00 pm, and 4:00 pm, temperature measurements were gathered three times daily. Weather, air quality, and water temperature were also noted. For three weeks, the experiment was carried out again. The water hyacinth was checked after a week had passed after the polythene sheets were removed, and it revealed serious injuries but was not entirely harmed. According to the statistics, the number of water hyacinths population significantly decreased, and after one week, nearly 90% of the plants began to recover. After two weeks, the plastic sheets were taken off. Significant damage was seen, although it seems that weeds recovered by 10% in the week that followed the sheet removal. After three weeks, they eventually saw that the plants had fully decomposed and were completely dead.

Photo 7: Effect of Solarization on water hyacinth

Chemical Control

Glyphosate and 2, 4-D are efficient weedicides for controlling water hyacinth (*Eichhornia crassipes*) (Fawad et al., 2015; Labrada, 1996). Glyphosate was used to treat water hyacinth (Weidong et al. 1999; Katembo et al. 2013). The use of 2, 4-D for the management of water hyacinth was described by (Carlock, 2003). (Chu et al., 2006) investigated the usage of the herbicides Roundup (Glyphosate) and 2, 4-D for the management of water hyacinth in China. They also mentioned KWH02, an environmentally benign weedicide that inhibits asexual reproduction and achieved 70% control of water hyacinth. According to Jadhav et al. (2008), glyphosate retardant levels applied at 0.8% work best for controlling water hyacinth while causing little to no impact to the aquatic habitat. However, chemical control contaminates water, thus if at all practical, a different strategy should be utilized for better management. The usage of water is halted for a large amount of time when chemicals are applied to water to manage aquatic weeds, which may upset the aquatic environment and have an impact on the habitats of native flora and wildlife. Particularly in poorer countries, it might pose a health danger to birds and native people. Use of AF101, a specific formulation with reduced mammalian toxicity, is recommended in urgent situations (Parsons and Cuthbertson, 1992).

Photo 8: Effect of herbicide on water hyacinth

Photo 9: Effect of herbicide on water hyacinth

Integrated Management of Water Hyacinth

Laws and regulations for controlling water eutrophication and developing a surveillance system, coordinating governmental agencies, managing watersheds, and using resources should all be in place to avoid the invasion of water hyacinths. For the control of water hyacinth, there is a dire need to adopt an integrated management strategy that included human removal, mechanical removal, allelopathy, biological control, and solarization. The combination of all these management strategies will efficiently reduce water hyacinth populations, but the right timing and environmental factors should be considered when choosing the best strategy to achieve maximal control at the lowest possible cost. According to Jianbo et al. (2007), the effective management of water hyacinth requires the integration of several control strategies with usage and landscape-level adaptive management.

CONCLUSIONS

It is not simple to establish a specific water hyacinth management program for developing countries of the world. Socioeconomic factors may make it impossible to use a specific control method. These factors include public opinion, which is often influenced by the nation's scientists, who will legitimately raise concerns about any introduction of chemicals or bioagents, the use of any control measures, and a lack of resources, funding, and trained personnel. Additionally, there could not be an appropriate connection between the concerned national institutions. The need to develop control techniques based on the extent of water hyacinth infestations and national socio-economic restrictions must coexist with the need to continue studies to enhance water hyacinth control. Without a question, water hyacinth is the most problematic aquatic weed in tropical and subtropical areas of the world. The economics and environments of the countries are severely harmed by its occurrence in water bodies. As a result, logically conceived and implemented weed control systems should be technically sound.

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CHAPTER 5

ORGANIC BEEKEPING AND THE USE OF NATURAL PRODUCTS IN COMBATING WITH VARROA PARASITE IN ORGANIC BEEKEPING*

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INTRODUCTION

Organic beekeeping is defined as beekeeping activities performed in the natural and unspoiled areas or organic farming areas to produce bee products without any artificial nutrition and chemical spraying at all stages from production to consumption. (Gökçe ve Konak, 2003). For bee products to be accepted as organically produced, the characteristics of hives, the quality of the environment, the conditions in which bee products are obtained, processed and stored must comply with the standards of organic farming. For this reason, when there are agricultural activities performed in the same area with an organic beekeeping farm, for the products of this beekeping farm to be accepted as organic, the agricultural activities must comply with the standards of organic production (Anonim, 2002a) because harmful substances emitted from external sources pollute water, soil and air, and agricultural activities in the same environment are also affected. Thus, the agricultural products themselves are damaged and the colonies in the same environment are damaged as well. In other words, organic bee farming should be done in agricultural areas where organic farming is applied. Though varying from community to community, animal and plant health and environment protection awareness has made great strides in general. Producers and consumers are increasingly turning to agricultural products that are produced by methods that do not destroy nature, and that do not cause toxic effects on humans (Anonim, 2002a).

The current and favorable geographical structure in our country and the vegetation that is integrated with this geographical structure provide a very suitable environment for the production of organic bee products (Konak, 2003). It is possible to produce organic honey and pollen especially in pine forests, in large-scale pasture areas, in nectar-blooming areas where acacia, chestnut and lime trees grow (Gökçe, 2002). However, these conditions are not sufficient for organic production and breeding conditions are also important. One of the obligatory conditions for transition from traditional beekeeping to organic beekeeping is to meet the needs of beekeepers who pass to organic beekeeping and to keep infrastructure wastes released and the honeycombs in the colony under control. It is one of the essentials of organic beekeeping to meet the demand of beeswax, which is highly demanded by beekeepers and is not found in the desired quality (Imdorf et al., 2003; Lodesani at al., 2003; Livia et al.; 2003; Şahinler 2013).

It should not be forgotten that the main purpose when fighting diseases is to protect bee health. If we want productive beekeeping and healthy bee products, it is necessary to apply treatments in certain periods. During treatment applications, medicines should not leave residues in honey products especially in honey and beeswax. Consumers in developed countries have begun to prefer residue-free products. With the use of organic drugs in diseases and pests, diseases can be controlled more easily and the problem of residue can be eliminated. Moreover, the use of organic drugs will also help reduce the amount of toxic elements on bee products (Demirhan ve Sahinler, 2016).

1. PROTECTION OF HEALTH ACCORDING TO THE STANDARDS OF ORGANIC BEEKEEPING

Like all living things, honey bees are under the threat of various parasites and microorganisms in the environment. These parasites and microorganisms cause the bees to become sick, colonies to fall weak and fragile or to collapse, thus reducing their yield. While in conventional beekeeping, chemicals are often used to control diseases and pests, in organic beekeeping, necessary precautions are taken to prevent diseases or products or methods that do not harm organic production are preferred. In this regard, the first thing to be done is to take preventive measures to protect bees against diseases. To do so;

1. Tough races and lines should be selected and queen bees should be regularly renewed
2. The male hatchlings should be controlled by systematically monitoring the hives
3. Materials used in bee yards should be disinfected regularly with organic methods
4. Contaminated materials or sources must be disposed of in a harmless way
5. Honeycombs should be regularly renewed
6. Sufficient amount of pollen and honey should be left in the hives (Gökçe, 2002).

Disinfection of hives must be done by burning with blowtorch. Other beekeeping materials should be disinfected in boiling water. In order to disinfect hives and equipment used in beekeeping; sodium hydroxide, potassium hydroxide and sodium soap, water and steam, lime slurry, lime, quicklime, sodium hypochlorite (e.g. bleach), caustic soda, caustic potash, oxygenated water, natural plant extracts, citric, paracitic acid, formic acid, oxalic acid, acetic acid, alcohol, formol, and sodium carbonate can be used (Anonim, 2002b; Losedani et al, 2003).

Despite all precautions, if colonies get sick, treatment should be initiated immediately and the medicines should be applied according to organic production rules. Transition period is applied to the colonies taken into treatment and they are transferred to organic honeycomb frames. If chemical medication is inevitable, it should be done at the veterinary control. After the treatment, all the honeycombs in the colony where the medication is done should be replaced with new honeycombs. When, one of the biggest problems in beekeeping, Varroa destructor parasite is seen in a colony, then formic acid, lactic acid, acetic acid, oxalic acid, menthol, thymol, eucalyptol and camphor should be used (Anonim, 2002b; Gökçe, 2002; Imdorf et al., 2003; Livia et al.; 2003).

As an example of drug use in the production of organic honey, the use of salt instead of naphthalene against candle moth and the use of formic acid against varroa and lime disease can be given (Kumova ve Korkmaz, 2000). Because formic acid is a naturally occurring organic acid, in proper dosage it does not leave any residue on honey and does not adversely affect human health due to the fact that the ratio of formic acid is reduced to natural limits shortly after application, it can be used as preparate to combat with Varroa and lime disease (Kaftanoğlu ve ark, 1992).

Apart from this, male bee hive frames can be used as biological method. Another method of combating is the heating method. In this system, the hive is heated at 45 0C for 5 minutes. The Varroas falling off to the bottom of the hive are collected and destroyed (Gökçe, 2002).

Varroa destructor is a dangerous parasite living on larvae, pupae and adults of honey bees (*Apis Mellifera L.*) by sucking their blood. It main host is *Apis Ceren*, also known as Indian bee. This parasite was infected with *Apis Mellifera* as a result of migratory beekeeping for more honey production(Kaftanoğlu, 2000). The Varroa destructor, which first went to Russia and then to the Eastern European countries, then spread to all the continents and caused the collapse of hundreds of thousands of colonies. It first came to Turkey in 1977 and spread to the whole country in a short time. Although many measures have been taken, it still poses a great threat to colonies (Kaftanoğlu ve ark., 1990; Kaftanoğlu ve ark., 1992; Kaftanoğlu ve ark., 1995; Tutkun ve İnci, 1992). The adult Varroa is brownish or dark brown and covered with a chitin layer throughout the body. Adult females are 1.1-1.2

mm in length and 1.5-1.6 mm in width (Genç, 1996; Kaftanoğlu ve Ark., 1995; Kaftanoğlu ve Yeninar, 2000).

Female Varroa normally does not have reproductive ability. In order to gain ovulation ability, it must be fed with the juvenile hormone in the hemolymph of the larvae. Therefore, a short time before the larvae is sealed, Varroa enters the honeycomb where the larva is and 60 hours after it has taken enough juvenile hormone begins to lay eggs. In one male bee honeycomb cell, 5 female Varroas and in one worker bee honeycomb cell, 3 female Varroas can grow into adulthood. After coming out of the honeycomb cell, Varroas cling to any bee inside the hive and feed on its blood (Kaftanoğlu ve Ark, 1990; Kaftanoğlu ve Yeninar, 2000; Tutkun ve İnci, 1992).

As a result of *Varroa destructor* 's feeding on blood, bees lose protein; thus, bacteria, fungi and other pathogens can easily enter the body. Microbial infection and loss of protein shorten the life span of bees and greatly increase the wintering losses. The flying activities of those which can survive become very low. Bees affected from the Varroa mite when they are in their pupa period receive more harm and can complete their development with some deformations such as without wings, with only one wing, with a small abdomen and with small feet (Kaftanoğlu ve ark.1990).

In the control of the Varroa parasite, biologic control methods are used. These biologic methods include reduction of the production of male honeycomb cells, transportation of the cells in which Varroa mites exist and trap method, trapping method by taking artificial swarms, bottom application method with wire net and drawers, the method of applying electricity to honeycomb wires, the method of using young queens, making use of heating applications, using pollen trap, changing the size of worker bee honeycomb cell (Akyol ve Korkmaz,2006).

MATERIALS AND METHOD

The research was carried out on 28 honey bee (Apis Mellifera L.) colonies.The study was carried out in a single stage, in 4 groups and 7 colonies of equal strength in each group.

1st Group: It was prepared from tobacco leaf as 50 gr. and was given in the form of smoke five times after it had been burnt in the smoker. This treatment was repeated 10 times at three-day intervals. The Varroas falling onto the hive drawer 15 hours after the tobacco leaf treatment were counted daily.

2nd Group: 10 gr. thyme oil was burnt in the smoker and then was given in the form of smoke five times with the smoker. The thyme oil treatment was repeated 10 times at three-day intervals. The treatment was performed in the evening time when bees come back to their hives and after the treatment, the entry to the hive was kept closed for 15 minutes. The Varroas falling onto the hive drawer 15 hours after the thyme oil treatment were counted daily.

3rd Group: 10 gr. bay oil was burnt in the smoker and was given in the form of smoke five times. The treatment was repeated 10 times at three-day intervals. The Varroas falling onto the hive drawer 15 hours after the bay oil treatment were counted daily.

4th Group: For the control group which was not going to be subjected to any disinfection, only sawdust was burnt in the smoker.

The study was conducted on 28 honey bee (*Apis Mellifera L.)* colonies. The study was conducted as a single replicate including 4 groups in each of which there were 7 colonies in equal strength.

Before the study, two honeycombs were taken from each colony to determine the level of Varroa septicity in the honeycomb cells where there were baby bees; from both faces of the honeycombs, larvae were taken. On each face of a honeycomb, 50 closed baby cells with the size of 30 cm^2 (6x5 cm); thus, a total of 200 cells were opened in each colony and the Varroas here were calculated. In the closed cells where there are baby bees, the varroa septicity was calculated (%) with the following formula.

$$KAGV\ (\%) = \frac{NV}{NKAG} \times 100$$

KAGV= varroa septicity (%) in the closed cells

NV= The total number of varroas in the opened cells

NKAG= The number of the closed cells which were opened

In the colonies, the level of total varroa septicity (%) before and after the treatment was calculated with the following equation[19].

Bcolony= The total % varroa septicity value of a bee colony

MV = The number of varroas calculated on an adult bee

Me = The number of the adult bees sampled (150-200 bees)

Ma= The total number of bees in the treated hive

Nv = The number of varroas in an open baby cell

Ny = The number of the closed baby cells which were opened (the number within a 30 cm^2 area)

Na = The number of total closed cells of the colony (the number of cells within the area where there are baby bees)

Moreover, 50 adult bees taken from each hive were killed in detergent water and the septicity ratio in these bees was calculated with the following formula.

$$\text{EAÜV (\%)} = \frac{\text{NEAÜV}}{\text{NEA}} \times 100$$

EAÜV= varroa septicity (%) on an adult bee

NEAÜV= The total number of varroas on an adult bee

NEA= The total number of adult bees

All the values obtained at the end of the study were calculated according to Henderson-Tilton formula (Karman, M.,1971).

$$\text{Effect value (\%)} = \left(1 - \frac{\text{Post-treatment septicity (\%) x The last septicity of the control group (\%)}}{\text{Pre-treatment septicity (\%) x The first septicity of the control group (\%)}}\right) \text{X100}$$

RESEARCH FINDINGS AND DISCUSSION

According to the treatments made in the autumn and spring periods and the findings obtained in the study;

Table 1. Results in the autumn term

	Pre-treatment Varroa septicity ratio (%)	Post-treatment Varroa septicity ratio (%)	Effect value (%)
1st Group(Tobacco)	49,33	25.20	35.55
2nd Group(Thyme)	46.67	32.80	12.30
3rd Group(Bay)	24.67	15.44	21.90
4th Group(Control)	38,67	48.25	-
Mean	39.83	30.42	23.25

As shown in Table 1, the tobacco leaf treatment in the autumn was found to be more effective than the others in combating with Varroa (35.55%).

Table 2. Results in the spring term

	Pre-treatment Varroa septicity ratio (%)	Post-treatment Varroa septicity ratio (%)	Effect value (%)
1st Group (Tobacco)	49,33	46.4	28.48
2nd Group(Thyme)	46.67	50.4	17.90
3rd Group(Bay)	24.67	32.12	0.52
4th Group(Control)	38,67	29.4	-
Mean	39.83	39.58	15.63

As can be seen in Table 2, the tobacco leaf treatment in the spring was found to be more effective than the others in combating with Varroa (28.48%).

As a result of the study in which the eucalyptus bark and leaf and the orange peel were used against Varroa, it was found that the eucalyptus bark and leaf group was found to be 94% effective and the orange peel group was found to be 99% effective (Çetin,2010).

The effects of formic acid plaques on lime disease caused by *Varroa destructor* and *Ascosphere apis* were investigated and its bioactivity against *Ascosphere apis* was determined in vitro conditions. Formic acid application to colonies was carried out 4 times in 4 days intervals in the spring of the same year. The efficacy against *Varroa destructor* was found to be 93.3% on average and against lime disease was found to be 45.28%. It has been reported that doses of formic acid administered to colonies do not have a negative effect on queens, worker bees and colony development (Kaftanoğlu ve ark.1990).

While the European Union established organic agricultural production standards, it took medicines and various chemicals used to combat with Varroa under surveillance in order to ensure unity between Member States. Scientific studies on these substances have suggested the use of formic acid, lactic acid and oxalic acid. It has also been reported that alternating drug use (e.g. formic acid in the early spring and oxalic acid in the autumn and lactic acid in the other spring) is also highly prevalent in preventing varroa resistance to chemical agents (Akyol and Özkök, 2005).

In one study, the effectiveness of juniper tar smoke against *V.destructor* was found to be 3.61% (± 4.51) on average and the effectiveness of cardboard was found to be %2.64 (±0.78) on average. The variance analysis conducted on the basis of the calculations made for the numbers of varroas falling onto the drawer as a result of smoke treatments revealed that there is no significant difference between the treatment and control groups ($p>0.05$); thus, juniper tar smoke was found to be ineffective against *Varroa destructor* (Girişkin ve ark.2007).

In a study conducted in Erzurum, the effects of oxalic acid, thymol and lactic acid on Varroa destructor infestation activity and colony development were investigated. As a result of the experiment, the efficacy of oxalic acid, thymol and lactic acid against Varroa destructor infestation was determined as 84.90 ± 5.60%, 90.10 ± 3.03% and 79.50 ± 3.78% respectively. According to the results, the difference between the efficacy scores of the organic compounds used for Varroa destructor was statistically significant ($P < 0.05$). It was also found that the number of adult bees dying after the oxalic acid group treatment was significantly different from thymol and lactic acid groups ($P < 0.05$) (Cengiz, 2012).

The activity of Apivar (Amitraz) was investigated in the honey bee colonies (pollen-trapped hives) naturally infected with Varroa destructor in Bursa region. In the pollen drawers found in the Apivar group, a total of 8838 Varroas fell off within a 42-day period and 57% of them fell off within the first 48 hours. In the control group, a total of 1923 Varroas fell off in the pollen drawers in the control group and 13% of them fell off in the first 48 hours. The effectiveness of Apivar was first determined with Henderson–Tilton formula and then with the percentage change method based on the principle of comparing of the mean percentages of the mites obtained before and after the treatment. Through these methods, the effectiveness of Apivar was found to be 99.43% and 99.36%. No side effect resulting from the treatment was observed (Aydın,and Girişkin, 2010).

In a study conducted in Hatay, Varroa parasite was treated with orange peel, eucalyptus leaf and bark were applied against Varroa parasite and that orange peel application was found to be more effective than other applications in autumn and spring followed by the application of eucalyptus bark and leaf mixture. The effectiveness of the treatments made in the autumn period was found to be higher than those made in the spring period (Sahinler, 2010). The findings of the current study also concur with those reported by similar studies in the literature.

In another study conducted in Hatay, a sufficient amount of application of powdered sugar into the hive at appropriate periods against varroa parasite was carried out and it was aimed to reduce the number of V. destructor naturally. V. destructor counts were performed before and after the trials. The effectiveness of powdered sugar application was determined as 39.72% in the morning and 44.26% in the afternoon by applying the Henderson-Tilton formula, which evaluates the difference in the number of mites on the bees before and after the treatment. There was no significant difference between these two levels of activity compared ($p<0.05$). It has been reported that application of powdered sugar, which does not carry the risk of residue and resistance, contributes to organic control in cases where varroa destructor infestation and nectar flow periods need to be combated.(Muz, et all.2014)

In the study titled İzmir Province Beekeeper's View on Bee Diseases and Pests, it was concluded that the most important problem beekeepers face from bee diseases and pests is varroa. Beekeepers say that 43.5% of the problem of drug residues in bee products is with organic drugs. They reported that 29.0% could be solved with education and 17.7% with the correct use of drugs. This result is consistent with the study (Kösoglu, M et al., 2019).

CONCLUSION

One of the most important factors preventing the development of our beekeeping is bee diseases and pests. Therefore, beekeepers should have information about the symptoms and characteristics of the most common parasites and diseases and the methods of combating with them. Unconscious and wrong practices will cause both economic loss and spread of disease to healthy colonies.

Complete elimination of Varroa, which lives on adult bees or in closed baby bee cells, is not possible with existing methods. It is necessary to reduce Varroa population in hives in order to be able to run effective beekeeping and to produce healthy bee products.

For this purpose, it is a necessity to apply continuous treatment at certain periods. To do so, physical, biological, genetic and chemical methods should be used in a harmonious combination. From among these methods, chemical ones are the least labor-intense, the least expensive and the easiest to apply. The current study was performed to encourage the use of natural products rather than chemical methods.

As a result of this study, it was determined that the effect of tobacco leaf, bay oil and thyme oil is higher than the control group in the combat with Varroa by 35.55%, 21.90%, and 12.30% respectively in the autumn period. In the spring term, the effect of tobacco leaf, bay oil and thyme oil was found to be higher than the control group by 28.48%, 0.52% and 17.90%, respectively. As a result, it has been determined that tobacco leaf application is more effective than the other applications in autumn and spring months in combating with Varroa and it is followed by the thyme oil application.

In the current study, and natural products were used against the Varroa parasites in the honey bee colonies within the context of good agricultural practices and organic agriculture. Thus, both pesticide residues are prevented from being left on honey and natural products for combating with Varroa parasites can be offered for beekeepers who make organic beekeeping.

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CHAPTER 6

THE EFFECTS OF GLOBAL WARMING ON FARM ANIMAL PRODUCTION AND THE EFFECTS OF FARM ANIMALS ON GLOBAL WARMING

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INTRODUCTION

Global climate change occurs as a result of global warming and affects our lives negatively every day. Climate changes is a result of changes in the composition of gaseous components in the atmosphere due to temperature increase. Climate change is felt more intensely in the Mediterranean with the significant decrease in precipitation in warm climates. This situation is directly proportional to the change of direction of the Atlantic wind towards the north. It is also predicted that there will be a significant warming, mostly in the summer season. Studies with regional climate change models show that the Mediterranean may be a region sensitive to global change (Giorgi and Lionello, 2008).

As a result of global warming; freshwater resources are drying up, species are disappearing, glaciers are melting, and sea levels are rising. Unfortunately, our country is one of the countries that will be particularly affected by the consequences of global warming. There are serious concerns that summer and winter temperatures will increase, precipitation will decrease, water levels in our seas will increase and agricultural production will decrease (Varol and Ayaz, 2012). For example, the Central Anatolia and South East Anatolia regions, which are under the threat of desertification more than the temperature increase, and the semi-humid Aegean and Mediterranean regions, which do not have enough water, will be more affected. It is predicted that climate changes will lead to changes in the natural habitats of animals and plants, and significant problems will arise in terms of water resources, especially in our regions mentioned above (Öztürk, 2002).

Global warming, unfortunately, may cause living species to leave the area they live in; Unfortunately, it can also allow new invasive species that are not native to the region to settle in that region. In addition, global warming causes some living species to disappear from the world (Doğan et al., 2015).

Animal production has been seriously intensified as a result of efforts to close the animal protein deficit. As a result of practices aimed at increasing productivity in animal production, negative effects on the environment have emerged and eyes have turned to animal production with the problem of global warming. animal production; It can lead to negative effects on many features of the environment such as decrease in soil quality, climate change, air-water pollution, and decrease in biodiversity. In a report prepared by FAO (United Nations Food and Agriculture Organization), animal production; reported that it is the main factor in climate changes and global warming (Steinfeld et al. 2006). In addition, it is stated in the same report that egg, milk, meat production is responsible for approximately 18% of human-induced greenhouse gas production, and this rate is higher than that produced by vehicles used by humans (Steinfeld et al., 2006).

Greenhouse gases in the climate system trap the long-wave infrared rays that are reflected back into space, causing the atmosphere to warm up. Greenhouse gases occur naturally in nature and emerge as a result of various activities of people (Atalık, 2005).

Gases that have an effect on global warming are methane (CH4), carbon dioxide (CO2), and nitrous oxide (N2O). Carbon dioxide (CO2) is the second most abundant greenhouse gas and is included in the atmosphere as a result of many natural events such as the respiration of animals and humans, the decay of organic matter, and volcanic eruptions.

In addition; The burning of fossil fuels, solid wastes, wood and wood products for transportation, heating and electricity production also causes an increase in the amount of CO2 released into the atmosphere. From the Industrial Revolution to the middle of the eighteenth century, the amount in the atmosphere has increased by 31% from 281 ppm to 368 ppm (Atalık, 2005).

Methane (CH4) is a gas that creates a more effective insulating gas in the atmosphere. It is included in the atmosphere during the production and transportation of oil, coal and natural gas. Methane occurs as a by-product of the digestive systems of animals, especially bovine and ovine animals, as well as the decomposition of organic materials in waste areas (Atalık, 2005). Unfortunately, the amount of methane gas in the atmosphere has more than doubled since the Industrial Revolution. Nitrogen monoxide (N2O) is formed as a result of burning fossil fuels by processing agricultural soils. It is also a very strong insulating gas. It was determined that the amount in the atmosphere increased by 17% when compared to the pre-industrial level (Atalık, 2005).

1. EFFECTS OF ANIMAL PRODUCTION ON GLOBAL WARMİNG

In recent years, it has been determined that industrial and intensive animal production has developed 2 times faster than traditional integrated livestock production and 6 times faster when compared to extensive livestock production based on pasture grazing (Verge et al., 2007). Aiming to meet the demand for animal products, the capacity of the animal production sector is expected to double by 2050 (Steinfeld et al., 2006). As a result of the intensification of animal production, the increase in the size of the herd, the negative practices in animal welfare, the low availability of land in the densely populated areas, the inability to use fertilizers correctly, the insufficient lands of intensive livestock enterprises or their inability to compete with other branches of agriculture, high-input.The ineffective use of nutrients in production at both animal and soil level increases the amount of dangerous emissions (such as phosphorus, nitrogen, methane, CO2) (Görgülü et al 2009).

1.1. The Effect of Produced Greenhouse Gas on Global Warming

Greenhouse gases contribute very little (<1%) to the earth's atmosphere. However, these gases play an important role in maintaining the atmospheric temperature of the ecosystem formed by humans, plants and animals. The anthropogenic effects, which are the main source of the increase in greenhouse gases; 49% energy use; 24% industry; 14% reduction of forests and 13% agricultural activities. As a result of such activities, greenhouse gases such as CO2, CH4, N20, CFC accumulate in the atmosphere and cause climate change based on unnatural, anthropogenic factors (Türkeş, 2007).

1.1.1.Effects of Cattle Breeding

Greenhouse gas emissions from cattle account for approximately 65% of the total emissions from the livestock sector (4.6 billion tons of CO2 equivalent). This result shows that most of the greenhouse gas produced as a result of livestock activities originates from cattle. Meat production in cattle accounts for 41% of total greenhouse gas emissions, while milk production includes 20% of greenhouse gas emissions. Emissions from other goods and services are 0.3 billion tons (Table 1). While the energy requirement for the production of feed raw materials used in animal nutrition constitutes 10% of the total CO2 emissions, the amount of energy consumed in meat and milk production is considered insignificant in emissions.

Table 1. Cattle meat and milk production, emission amount and emission intensity (Anonymous, 2013)

Production Type	System	Production (million tons)		Emission (million tons, CO2)		Emission Intensity (kg CO2 equivalent / kg product)	
		Milk	Meat	Milk	Meat	Milk	Meat
Milk	Pasture	77.6	4.8	227.2	104.3	2.9	21.9
	Mixed	430.9	22.0	1104.3	381.9	2.6	17.4
	Total Milk	508.6	26.8	1331.5	486.2	2.6	18.2
Meat	Pasture		8.6		875.4		102.2
	Mixed		26.0		1462.8		54.2
	Total Meat		34.6		2338.2		67.6
Product Handling				87.6	12.4		
Total		508.6	61.4	1419.1	2836.8	2.8	46.2

1.1.2. Effects of Buffalo Breeding

As can be seen in Table 2, greenhouse gas emissions resulting from water cattle production constitute 9% of the total emissions. This value is equivalent to 390 million tons in milk production, 180 million tons in meat production, together with other goods and services, in total 618 million tons of CO2.

Enteric fermentation and forage crop fertilization; While enteric fermentation in milk production and cattle manure occurs over 60%, this rate is around 45% in cattle. When the ratios obtained are examined, this difference is due to the digestibility of the feeds. Fertilization used in the production of forage crops is the second largest emission source, with 21% in meat production and 17% in milk production.Tablo 2. Mandalarda süt ve et üretimi, emisyon miktarı ve emisyon yoğunluğu (Anonim, 2013)

Table 2. Milk and meat production, emission amount and emission intensity in buffaloes (Anonymous, 2013)

System	Production (million tons)		Emission (million tons, CO_2)		Emission Intensity (kg CO_2 equivalent / kg product)	
	Milk	Meat	Milk	Meat	Milk	Meat
Pasture	2.7	0.1	9.0	4.7	3.4	36.8
Mixed	112.6	3.2	357.9	175.2	3.2	54.8
Product processing			23.0	0.3		
Total	115.3	3.3	389.9	180.2	3.4	53.4

1.1.3. Effects of Sheep and Goat Breeding

Greenhouse gas emissions from sheep and goats constitute 6.5% of the total emissions. As can be seen in Table 3, approximately 299 million tons of meat, 130 million tons of milk, and 475 million tons of CO_2 equivalent emissions are generated from sheep and goats, together with other goods and services. Generally, goats have a lower emission intensity in milk production than sheep due to higher milk yield than sheep. In terms of emission intensity in meat production, there is no big difference between sheep and goats.

Forage crops fertilization and enteric fermentation; More than 55% of the emissions from meat and milk from sheep and goats are caused by enteric fermentation. More than 35% of this determined value is due to feed production. It is thought that leaving the manure in the pasture is effective in the lower manure emissions in sheep and goats compared to other animals.

Looking at the relationship between wool production and emissions; In regions that stand out at the point of natural source wool production and provide high income, a significant portion of these products are beneficial in reducing the share of emissions originating from milk and meat production on the basis of emission emissions. Globally, it has been determined that 45 million tons of CO_2 is produced as a result of wool production in the world.

Table 3. Production, emission amount and emission intensity of sheep and goats (Anonymous, 2013)

		Production (million tons)		Emission (million tons, CO_2)		Emission Intensity (kg CO_2 equivalent / kg product)	
Type	System	Milk	Meat	Milk	Meat	Milk	Meat
Sheep	Pasture	3.1	2.8	29.9	67.3	9.8	23.8
	Mixed	5.0	4.9	37.1	115.0	7.5	23.2
	Total	8.1	7.7	67.0	182.3	8.4	23.4
Product processing				0.3	4.1		
Goat	Pasture	2.9	1.1	17.7	27.2	6.1	24.2
	Mixed	9.0	3.7	44.3	84.5	4.9	23.1
	Total Milk	11.9	4.8	62.0	111.7	5.2	23.3
Product processing				0.4	1.0		
Total		20.0	12.5	129.7	299.1	6.55	23.85

1.1.4. Effects of Poultry Breeding

Emissions arising from chicken production in the world are 606 million tons of CO_2 and this amount represents 8% of the emissions in the sector (Table 4). Its contribution to chicken meat and egg production in feed production is approximately 57%. Approximately 30% of the emission values are caused by manure management, and 7% of meat production and 20% of egg production are composed of manure residues and synthetic fertilizers.

Table 4. Chicken meat and egg production, emission amount and emission intensity (Anonymous, 2013)

System	Production (million tons)		Emission (million tons, CO_2)		Emission Intensity (kg CO_2 equivalent / kg product)	
	Egg	Meat	Egg	Meat	Egg	Meat
Pasture	8.3	2.7	35.0	17.5	4.2	6.6
Cage	49.7	4.1	182.1	28.2	3.7	6.9
Broiler		64.8		343.3		5 .
Total	58.0	71.6	217.0	389.0	3.7	5.4

Ways to reduce methane emissions in farm animals are also stated (Naqvi and Sejian, 2011);

1. Improving animal nutrition with quality and strategic supplementation of essential nutrients

2. To develop low methane producing animals in genetic selection.
3. Reducing the roughage rate in their rations and increasing the concentrate feed rate
4. Improve pasture management and use
5. Providing animals with appropriate care and health conditions
6. Reducing methane production by changing the diet towards ammonia and molasses
7. Destruction of protozoa in the rumen and microbial intervention in the rumen
8. Producing roughage and pasture forage crops that emit less greenhouse gases
9. Using alternative fodder plants and concentrates with high tannin and saponin content
10. Reducing the production of animal products
11. Developing recombinant and immune technologies
12. Reducing the number of animals by increasing productivity in animals
13. Adding vegetable oils to the ration
14. Using probiotics that will suppress methanogenic microorganisms and compete with them
15. Use of secondary plant components such as essential oils in animal nutrition

2. THE EFFECTS OF GLOBAL WARMING ON LIVESTOCK

The effects of global warming on animals emerge as the direct effects of the physical environment, biological environment, chemical environment or climate. Physical environmental conditions are manifested by the effects that will occur in the care and feeding conditions. Due to extreme climatic conditions, housing becomes more costly, and some performance-related features such as reproduction, milk and meat yield may decline. Various studies have shown that it is effective on milk quality and quantity and shortens the lactation period in dairy animals under thermal stress conditions (Beede et al. 1985, Chase et al. 1988, Bucklin 1991, Alnaimy et al. 1992). In studies conducted on reproductive performance, however, fertility decreased (Alnmier et al. 2002; DeRensis and Scaramuzzi 2003; Drew, 1999), prolongation of the first insemination period and a decrease in pregnancy rate due to the inability to detect estrus (Alnmier et al. 2002; DeRensis et al., 1999). 2002), a decrease in blood flow to the uterus due to increased body temperature due to thermal stress, and consequently a decrease in the fertilization rate, limited embryonic development and an increase in early embryonic deaths (DeRensis et al., 2002). In addition, the growth and development of offspring are adversely affected by these conditions (Koluman Darcan et al., 2009; Çoban et al., 2008, Darcan, 2005). Studies have shown that in livestock, loss of appetite, decrease in feed consumption, decrease in feed efficiency and fattening performance are adversely affected. (Davis et al. 2001, Göncü and Özkütük, 2003; Silanikove, 2000; Harner et al. 1999; Linn 1997)

Climate change is indirectly effective on issues such as the quality and quantity of feed used in animal feeding, seasonal availability of pastures, genetic studies, number of animals, and animal health. The quality and quantity of the feed given to the animals is important in the rations prepared. Grain feeds such as barley and wheat used as animal feed, oilseed residues such as cottonseed meal, sunflower seed meal make it possible to prepare rations at low cost without losing their nutritional value (Koluman Darcan et al., 2009).

Due to the decrease in water resources and plant production areas, pasture or forage crop production will be adversely affected. Since the areas where crop production can be made will decrease due to the rise in sea water level, drought or salinity, it will be planned to produce foods for human nutrition in the existing areas. The competitiveness of animal feed production will decrease due to economic reasons and priorities. As the precipitation regime changes, drought will occur in some of the agricultural lands and salinity will occur in others.

Studies on genetic improvement of animals reared in terms of yield characteristics and productivity are constantly on the agenda. However, it will be important to consider that domestic animals can maintain their productivity in all conditions and be advantageous against the negative effects of the environment. This is less affected and with the help of the mechanisms they have, they can easily remove the extra heat from their bodies and maintain their body temperature. Drought due to climate change, therefore, studies on genotype should be based on selection. Since characteristics such as life expectancy, animal health and productivity of domestic gene resources are also effective on greenhouse gas emissions, they will be able to maintain their advantages over hybrid breeds in this respect, even when it comes to climate changes. In addition, due to their anatomical and physiological structures, an increase in events such as floods and epidemic diseases due to thermal stress is predicted. For this reason, it is important to start using animals that are resistant to drought and diseases in production. Skin, hair type, sweat gland capacity, ability to reproduce and maintain efficiency in difficult conditions, resistance to diseases and parasites, metabolic heat production, tolerance to thirst, anatomical and morphological structure are important effects that may arise in climate changes (Koluman Darcan et al. et al., 2009).

In terms of animal health, some diseases will pose a significant threat to livestock in terms of treatment costs, yield losses and immunity in the future (blue tongue and gastroenteritis, etc.). Many effects such as thermal stress will play a role in the easier reproduction of disease vectors as a result of the decrease in the cold seasons, which are shortened to the emergence of these diseases and cause problems, which are above 15°C. The socio-economic effects of global warming, on the other hand, can be highly effective in the form of food insufficiency, especially in conditions where natural resources are limited (Koluman Darcan et al., 2009).

The expected changes in the global climate will change the behavior and life of honey bees. Increases in temperature and humidity in autumn will directly affect the development of honey bees and may cause problems in wintering (Şahinler et al., 2008).

Global food production is impossible without pollinators like the honey bee. However, in recent years, the worldwide honey bee population has been alarming with increasing mortality rates. These challenges faced by the honey bee raise the question of the future of our food supply. In addition, infectious diseases and parasites such as

Varroa destructor are one of the main factors in increasing honey bee mortality rates. Varroa destructor parasite reproduction is dependent on the honey bee brood and can destroy the entire colony. On the other hand, climatic changes can have a significant impact on honey bee breeding and mortality rates. Climatic weather events affect the vegetation and therefore the foraging possibilities of the honey bee (Switanek et al., 2015).

In beekeeping, the climate, which is one of the stress factors, directly affects the nutritional resources it needs, and for this reason, in our country, nomadic beekeeping is carried out to get maximum efficiency from the flora. Sudden climate changes; If precautions are not taken, it will result in unqualified queen production, decrease in resistance in weak colonies, vulnerability to diseases and pests, and eventually the extinction of the colony (Topal et al.,2016)

3. USE OF FEED ADDİTİVES AGAİNST HEAT STRESS CAUSED BY GLOBAL WARMİNG İN CATTLE

Thermal stress has a negative effect on milk yield in dairy cattle and on fattening performance in beef cattle breeds. In addition to the regulation of rations in the diet of cattle, there are "non-nutritive" additives that affect physiological mechanisms. These additives only work if the ration given to the herd is balanced. Otherwise, it cannot improve the conditions of animals exposed to heat stress (Varol et al, 2021).

Sodium bicarbonate is one of the most effective feed additives in heat stress. Increase the amount of dry matter intake by promoting cellulose intake in high-energy diets. Along with reducing the acidity in the rumen, it increases the digestive efficiency and dry matter intake (West, 1997). In some studies, Huber et al. (1994) reported that the use of fungal cultures (Aspergillus oryzae (AO) strains) reduced rectal temperatures in cattle. The use of 6 g niacin in cows under thermal stress resulted in an increase in milk yield of approximately 1 kg (Muller et al., 1986). It causes a positive effect under heat stress conditions. The oils to be used can be of vegetable origin (soybean, sunflower, etc.) or of animal origin. It contains 3% oil in the rations on a dry matter basis. This oil rate is approximately 5-6% with the use of oil seeds in the ration Exceeding these levels reduces the absorption of calcium and magnesium. There are studies showing that increasing the amount of vitamins A, D and E in the diet in cases of thermal stress will be beneficial (Gerald, 1999).Daily 100,000 IU Vitamin A, 50,000 IU Vitamin D It is recommended to use 500 IU Vitamin E doses.

Ruminant health is positively affected by additional additives to the ration, feed consumption, feed efficiency ratio, live weight gain, disease resistance, improvement in metabolic activities and as a result meat, milk and reproductive performance increase. In this way, stress-related yield losses are prevented and significant gains are obtained in terms of economic activities related to livestock, business profit losses, food supply and price stability.

CONCLUSION

In our country, which is under a great threat in terms of global warming and climate change, strategic plans for the future should be made by taking into account the scenarios and predictions put forward by scientists. Accordingly, some practices in the livestock sector, which is effective in greenhouse gas emissions, regional strategic planning and political and socio-economic structures for these are important in terms of minimizing the negative effects that will occur.

As a result of many effects such as changes in seasons, decrease in cold seasons above 15°C, easier proliferation of disease vectors and increase in atmospheric temperature; some diseases and treatment costs in livestock will increase and pose a significant threat in terms of yield losses. It will be important to take measures related to this issue as well.

Livestock is adversely affected by global warming and climate change. Changes in the climate are a big problem for the growers. Problems or planning that may arise in this regard should be done without losing time, sector stakeholders and relevant people should do the necessary work.

As a result, global climate change may have direct and indirect effects on the quality and quantity of feed given to animals, feeding plans, seasonal availability of pastures, genetic studies, number of animals, animal health. Preventing possible future effects of climate change on livestock systems will largely depend on the interactions of the components involved in this process. Transforming animal production into sustainable systems can significantly contribute to mitigating the effects of climate change. In this context, solutions that can be adapted at farm level in the short and long term can be suggested as follows (Anonymous, 2017);

1. Planning the planting dates of the business feed resources correctly
2. Taking technical solutions at the point of air-conditioning of the shelters at the point of protecting animals from extreme cold or heat
3. Selection of plant varieties that are suitable for the breeding demands of animals and water use, as well as for abnormal temperature and humidity conditions.
4. The use of plants suitable for these conditions with the help of the existing genetic diversity in animal nutrition and the new possibilities offered by biotechnology
5. Increasing the effectiveness of pest and disease control, for example better monitoring, diversified crop rotations or improving integrated pest management methods
6. Reducing water losses, improving irrigation practices, using water more efficiently and recycling or storing water
7. To improve soil management by increasing the water holding capacity to maintain soil moisture and to facilitate the maintenance of the vital activities of animals in the initiatives made in land management.
8. Introducing animal breeds that are resistant to thermal stress and adapting the ration models to be given to animals to relieve them under heat stress conditions.

It is expected that the global warming problem will decrease when the recommendations given are taken into consideration. Otherwise, it is expected that the negative effects of global warming on animals, and in parallel, on humans, will continue.

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CHAPTER 7

A STUDY ON THE EFFECTS OF DEVELOPMENTS IN THE EASTERN MEDITERRANEAN ON MARITIME TRADE *

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* This study contains citations from the author's unpublished doctoral thesis.

INTRODUCTION

Eighty percent of trade in the world is carried out by sea transportation, which is the easiest, most economical and safest mode of transportation. Due to the increasing production volume in China and Asia, the need for raw material demand makes maritime transportation more popular day by day, and it is predicted that the upward momentum in the maritime trade volume will continue due to this situation. As a matter of fact, maritime reports confirm this information with some estimation for future about maritime. For example, according to the report “Maritime 2050 Navigating Future” (www.gov.uk) published by the UK, by 2050 the global shipping industry will be handling much more cargo than it currently does. On the other hand, in the report "Global Marine Trends 2030" (www.lr.org) published by Lloyd's Register, it is predicted that container load capacities will reach 26,000 TEU and 450,000 DWT by 2030s.

The fact that the upward trend in maritime trade will continue brings up the issue of where these cargoes will be transported. When examined from this aspect, the North Pacific route is at the top of the routes with the highest freight flow in the world, the North Atlantic route is the second, and the Mediterranean route is the third (www.worldtradia.com). In other words, the Mediterranean constitutes the third route where the heart of the world maritime trade beats. The recent developments in the region, which is in an interesting situation in all aspects, have caused not only riparian countries but also non-riparian countries to be involved in many processes.

Maritime jurisdiction areas and the struggles for sharing these areas are at the forefront of the important problems that attract attention in the Eastern Mediterranean. In particular, the practices of Greece and the Greek Cypriot Administration (GCA) contrary to international law are dragging the problems in this field away from the solution and into deeper geopolitical problems. Although these problems seem to be related only to oil and natural gas reserves at first glance, it is obvious that some developments in the Eastern Mediterranean Sea areas are actually aimed at increasing the ownership activities of countries in these areas. However, Article 90 of the United Nations Convention on the Law of the Sea (UNCLOS) regulates the principle of "freedom of the high seas" by stating that "Every state, whether it has a coast or not, has the right to sail ships flying its own flag on the high seas" (www.un.org). Despite this, in practice, countries intervene in maritime trade for various reasons and with different methods and try to own the problematic sea areas in a way.

For all these reasons, within the scope of this study, the problems experienced in the maritime jurisdiction areas in the Eastern Mediterranean and the practices put forward by the countries due to their different perspectives on these problems and finally the possible effects of these practices on international maritime trade were examined.

1. PROBLEMS REGARDING MARINE JURISDICTIONS IN THE EASTERN MEDITERRANEAN

The Eastern Mediterranean, where the Turkish Republic of Northern Cyprus (TRNC) has many rights as a riparian, has a great importance in terms of geopolitical and geostrategic, as well as energy and economy. It can be stated that the news about the rich oil and natural gas deposits in the Eastern Mediterranean in the press of the Greek Cypriot Administration (GCA) and Greece in 2001 constituted the beginning of the disputes regarding this area. Especially the practices of the GCA since these dates and the agreements it has made with neighboring countries constitute the basis of today's problems.

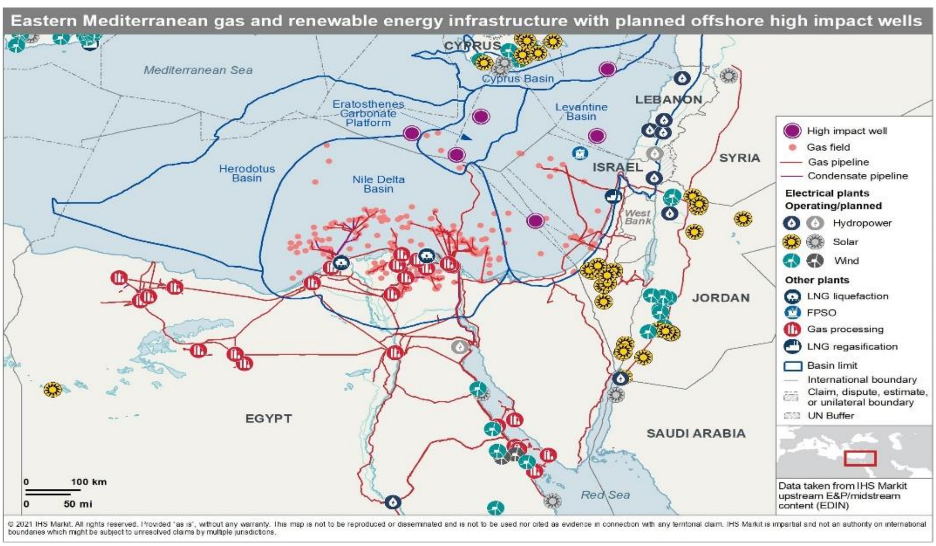

Figure 1: Eastern Mediterranean Gas and Renewable Energy Infrastructures
Source: https://ihsmarkit.com

The Eastern Mediterranean region including the Cyprus Island, is famous with hydrocarbon potential. According to the information given by the United States (USA) Geological Survey, as of 2010, there is an average of 3.45 trillion cubic meters of natural gas and 1.7 billion barrels of oil in the Levant basin, which is located in the middle of the states of Syria, Israel, Cyprus, and Lebanon. The presence of hydrocarbon resources is also mentioned in areas that are not covered. On the other hand, it was stated that the Nile delta basin in the north of Egypt has a larger estimated natural gas potential than many areas evaluated in the USA (www.aa.com.tr).

Shortly after the news about the Eastern Mediterranean, GCA signed exclusive economic zone agreements with Egypt in 2003, with in 2007, and with Israel in 2010. The GCA government has signed the mentioned agreements on behalf of a single country, by ignoring the TRNC. Actually, the areas subjected to agreement cover Turkey's possible continental shelf and exclusive economic zone areas, at every opportunity, Turkey always mention the objection to these agreements made by the GCA, both to the United Nations and to the relevant countries in UN (United Nations). (Acer, 2007, 2).

On the other hand, the exclusive economic zone areas declared by the Greek Cypriot Administration also ignore Turkey's maritime jurisdiction areas in the said region. Because the areas declared as if Turkey did not exist in the region also coincide with Turkey's drilling rights and exploration on hydrocarbon deposits. Especially since the oil and natural gas exploration permits granted by the GCA to other countries conflict with the areas to which Turkey has rights, it creates a constant tension in the region. Turkey, on the other hand, does not allow foreign ships to carry out exploration and drilling activities in the said area in every tension experienced in this way and tries to protect its own rights (www.insamer.com).

2. THE EFFECTS OF DEVELOPMENTS IN THE EASTERN MEDITERRANEAN ON SEA TRADE

The intensity of use of the Eastern Mediterranean, which is one of the important routes at the heart of world trade, tends to increase day by day in parallel with the increase in international trade. A visual of October 2022 from the www.vesselfinder.com page showing the locations of the ships confirms the density of ships in Eastern Mediterranean.

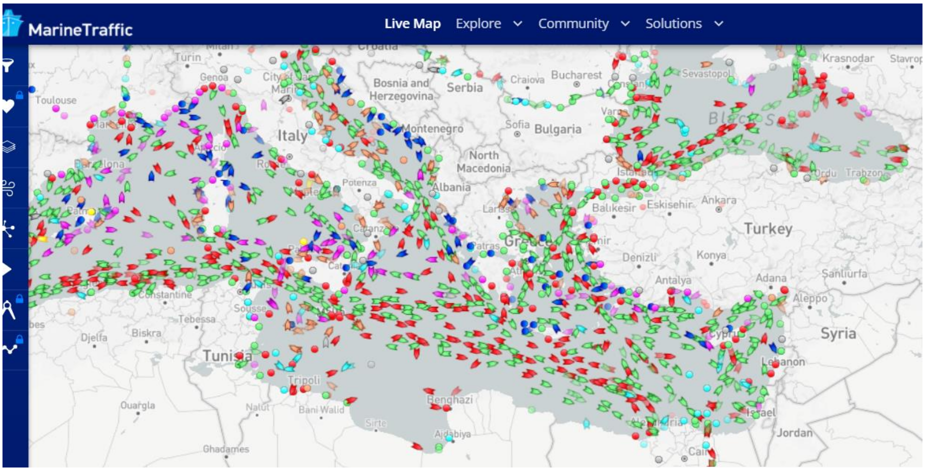

Figure 2. Eastern Mediterranean Ship Traffic
Source: https://www.vesselfinder.com

This density of maritime traffic also causes an increase in the interest in the region. In fact, practices such as transit passage and innocent passage in international law envisage the principles of freedom of the seas within the framework of certain rules. However, the practices regarding the use of the sea where the maritime jurisdiction areas are uncertain are remarkable in this respect. Because in a region where maritime jurisdiction areas have been formed, coastal states tend to determine their maritime trade routes. For this reason, the importance of controlling the sea routes due to both security and political and commercial transactions was a problem known to all states. In the Eastern Mediterranean, the sea's determination of its borders will be an important indicator of which countries will have which rights in these areas.

Although the regulations in the 90th article of UNCLOS regulating the rights of navigation are known by all the countries of the world, some developments in practice hinder maritime trade. Within the scope of this study, the efforts of countries to adopt maritime jurisdiction areas are explained with some case studies and practices. In particular, some of the case studies regarding the interrupting of ships sailing for various reasons in the Eastern Mediterranean are listed as follows (www.denizticaretgazetesi.org):

- **October 21, 2020:** A Turkish-owned and Panama-flagged ship was intercepted by the French frigate Latouche-Tréville, 135 miles from Benghazi, nothing suspicious was found in the search and the ship was allowed to continue on its way.

- **October 25, 2020:** A Syrian-flagged commercial ship was stopped by the French frigate Latouche-Tréville at a distance of 150 miles from Tobruk, as it only departed from the Turkish port. Cement to be used in construction works was found on the ship and the ship was released.
- **November 21, 2020:** A Panama-flagged ship departing from Egypt was intercepted at a distance of 42 miles from Tobruk and the ship was released after the inspection.
- **22 November 2020:** A Turkish flagged ship departing from Yarımca was stopped by a German frigate.
- **April 4 2021:** A Turkish-owned and operated Panamanian-flagged ship was stopped by France on the grounds that it was carrying "weapons or material to pierce the embargo to Libya" as part of the «Irini operation».

It is stated that these ships mentioned above were stopped within the scope of Operation IRINI. Operation Irene is a controversial operation launched by the European Union (EU) in the Mediterranean to oversee the United Nations' (UN) arms embargo on Libya. Launched on March 31, 2020, Operation IRINI (EUNAVFOR MED IRINI) is a European Union military operation under the umbrella of the Common Security and Defense Policy (CSDP). In particular, the mission is tasked with monitoring violations as well as conducting inspections of vessels in the high seas off Libya suspected of carrying Libyan-related weapons or related materials (www.operationirini.eu).

Considering that the ships inspected within the scope of Operation IRINI are predominantly Turkish-owned or Turkish-flagged departing from Turkish ports, suspicions arise that these operations are directed against Turkey. Unfriendly visits, especially within the scope of the IRINI operation, are not acceptable in terms of international law. Operations managers base these initiatives, which represent unlawful exit to ships, on the SUA (Suppression of Unlawful Acts against the Safety of Maritime Navigation) contract. Whereas, Article 8 bis-5 (d) of the Convention states that if a contracting state does not respond within 4 hours, a "boarding" authority will arise. However, there is a condition that it gives its notification to the IMO Secretary General. The State receiving a person in accordance with paragraph 3 may subsequently request the flag State to accept the surrender of several. The flag State shall consider such a request and act in accordance with Article

7 if it decides to accept it. If the flag State rejects this request, it shall notify the receiving State of the reasons (https://denizcilik.uab.gov.tr). In other words, the state that wants to do "boarding" is not authorized to board the ship, search and take the measures mentioned in the contract without the express consent of the flag state. Furthermore, according to Article 8 of the SUA Convention, “there must be reasonable grounds to believe that a crime has been committed.” In these interventions, how will it be known whether the reasons for stopping the ship are reasonable?

Another crucial issue regarding the stopping of ships in the Eastern Mediterranean is the European Union Common Fisheries Control Program in the Mediterranean. Within the scope of the program, seven member states; Fishery controls are carried out in the exclusive economic zone of the EU member states, by procuring resources and tools from the GAC, Greece, France, Italy, Portugal, Malta, and Spain. EU Common Fisheries Control Services also coordinates joint inspection activities carried out by vessels allocated by member states. The Italian ship, which took part in the control studies, operated in the Eastern Mediterranean between 7 – 28 November 2021 (in 3 periods, 7 – 12 November, 15 – 20 November and 23 – 28 November) (https://turkdegs.org). These activities, especially carried out in the Turkish Exclusive Economic Zone, are important in terms of influencing the processes of ownership of the seas.

One of the important matter that can affect navigation at sea is marine protected areas. Marine protected areas (MPAs) are mechanisms to protect specific areas of the sea. In recent years, countries, international organizations, environmental groups and other organizations have called for the establishment of MPA networks and for certain percentages of seas to be declared MPAs (www.gc.noaa.gov). Headquartered in France, MedPAN is a network of managers of Marine Protected Areas in the Mediterranean. To date, 8 founding members, 70 members and 49 partners from 21 Mediterranean countries have been involved in the process. Turkey is one of these executive countries.

Figure 3. Marine Protected Areas Management Implementation
Source: https://medpan.org/

The report published by the World Wide Fund for Nature Conservation (Figure 3) on marine protected areas reveals that the management of marine protected areas in the Mediterranean is extremely inadequate (https://medpan.org/). For this reason, Turkey should have a greater say in these areas, especially by declaring its Exclusive Economic Zone as soon as possible.

Another subject that has the potential to affect maritime trade is Particularly Sensitive Sea Area (PSSA). In particular, Identification of a PSSA is an internationally comprehensive management tool for reviewing the attributes of an area vulnerable to damage from international shipping and determining the most appropriate protective measures available through IMO. Along with the increasing oil pollution in the Eastern Mediterranean, the increase in the number of foreign species entering the region with ship ballast waters brings along the problem of ship-borne pollution. This situation reaches the extent of affecting climate change, which is described as a "hot topic", since it is one of the most crucial topics in the world recently. The main objective of declaring a PSSA is to provide protection from the risk posed by international shipping activities. The issue of additional measures to be taken (within the jurisdiction of IMO) for maritime safety and pollution prevention is important here. It is possible to control risks that may cause pollution, such as SOx emission control, and to impose special ballast discharge rules for ships sailing in this area. In this area, it is possible to control the risk posed by international shipping activities by implementing ship routing and reporting systems. Under this rule, all or part of a PSSA may be declared an "area to be avoided". Coastal states can indirectly affect international trade routes by identifying PSSA and activating mandatory guidance systems, ship routing systems or ship traffic management systems in this area, provided that the necessary criteria are met through an application to IMO (http://tudav. org).

Article 56, titled “Rights, Authority and Obligations of Coastal States in the Exclusive Economic Zone” of UNCLOS, paragraph 1, subparagraph (b) (iii), authorizes the coastal state to protect and preserve the marine environment in its exclusive economic zone. According to paragraph 5 of Article 211, coastal states, through the authorized international organization or diplomatic conference, adopt laws and rules in accordance with internationally generally accepted rules and principles for the prevention, reduction and control of pollution caused by ships in their exclusive economic zones (www.un.org). For this reason, coastal states have the right to take the necessary precautions and measures in especially sensitive maritime areas.

CONCLUSIONS

It is obvious that the Eastern Mediterranean, which has maintained its commercial vitality since the earliest times of history, will shoulder the burden of maritime trade with an increasing momentum in the future, depending on the volume of international trade. For this reason, the applications in the Eastern Mediterranean Sea areas are of interest not only to the riparian countries, but also to all the countries of the world using this sea area. Particularly in terms of Turkey, the issue of sharing sea areas by making agreements with riparian countries in the Eastern Mediterranean is of great importance.

In maritime trade, the safe arrival of the goods at the port constitutes the backbone of the trade, and the realization of the relevant transportation on the specified day and time is one of the most important elements of international competition. For this reason, delaying ships for even a few hours in maritime trade has unavoidable costs for both the carrier and the shipper. The interruption of the commercial activities of the ships, on the one hand, puts the companies in commercial losses, on the other hand, it brings along the security problems of the countries. Because the flag is an indication that a ship is under the protection of the state whose flag it is flying. In principle, ships are under the authority and control of the state whose flag they fly. To trespass on a Turkish flagged ship is to oppose the authority and control of the Turkish state, and this is completely against international law.

It is considered that all of the issues covered in this study, such as boarding within the scope of the IRINI operation, European Union Common Fisheries Control Program, Marine Protected Areas, Identification of Particularly Sensitive Sea Areas have high potential to affect maritime trade routes. In particular, the articles of UNCLOS on rights in the exclusive economic zone confirm this situation. Because when countries declare an exclusive economic zone, they have authority on many issues. In particular, Article 73 of UNCLOS titled “Implementation of the Laws and Regulations of the Coastal State” states that “the coastal state in order to exercise its sovereign rights in the exploration, operation, protection and management of living resources in its exclusive economic zone; may take all necessary measures, including approaching, controlling, arresting and adjudicating” (www.un.org). It regulates its provision and gives countries important rights in this regard. For all these reasons, it is both an important responsibility and an obligation for Turkey to declare its own exclusive economic zone in the Eastern Mediterranean.

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https://www.aa.com.tr

https://www.insamer.com

CHAPTER 8

IMPORTANCE OF AEROBIC RICE RESEARCH IN TURKEY: POTENTIAL, CHALLENGES, AND OPPORTUNITIES. A REVIEW

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INTRODUCTION

Rice is the most important crop after wheat, among other cereals as a food source. Besides, rice is the only type of cereal that can germinate in water, and its roots can use dissolved oxygen in the water. Rice is the main food for more than half of the people living in the world. Rice consumption represents 25% of the daily energy per person in the world (Seck et al., 2012). In particular, the protein content of rice is quite high compared with other cereals. Rice contains 5-10% protein in its composition, and there are about 4% lysine and threonine in rice, which amino acids are rarely found in other cereal sources (Amagliani et al., 2017). This makes rice the most widely used product after wheat to meet human nutritional needs.

In Turkey, rice is the most important grain to meet the food needs of wheat. Historically, rice farming in Turkey is known to have existed since 500 years ago. There is no conclusive evidence that when and where the first time rice farming was carried out. However, around the 15th century in the Anatolian region (as Turkey was known in the past), rice farming was very dominant in Kastamonu Province and Tosya District (Avcı, 2012; İbret, 2003; 2004). The rice seeds used are thought to have originated in Southern Egypt. According to records of the Ottoman Empire; In the 16th century, around 1719-1720, Tosya Regency had exported its rice production to other regions. Apart from Tosya, there are also rice fields in Anatolia, Beypazarı, Niksar and Boyabat (Kankal, 1991). It is also reported that rice production is being carried out at the bottom of the valley that runs along the Devrez River to obtain delicious rice for special sultan serving (İbret, 2003; 2004).

In fact, before the Republic of Turkey's proclamation, there were only a small number of rice fields in the Provinces of Kastamonu, Maraş, Diyarbakir, and Bursa (Şahin, 2002). However, the rice is only consumed by wealthy families in big cities. In 1926, three years after the Republic of Turkey's proclamation, the first rice processing plant was built in Tosya (İbret, 2003). Currently, Turkey cultivates rice in 7 geographic areas. The largest rice cultivation area is in the Thrace (Trakya) area of 44,790 ha, followed by the Black Sea (Karadeniz) area of 23,846 ha. Furthermore, the Marmara Region with an area of 21,259 ha, the Mediterranean Area of 5,941 ha, the Central Anatolia Region of 4,827 ha, the Southeast Anatolia Region of 1,689 ha, and the East Anatolia Region of 370 ha. Overall, rice cultivation is carried out in almost 32 provinces in Turkey, with Edirne Province as the highest rice producer, followed by Balıkesir Province and Samsun Province.

Rice production in Turkey is heavily dependent on irrigated rice fields, but its sustainability is threatened by the scarcity of fresh water, water pollution, and competition for water use. In Asian countries, irrigated lowland rice production consumes more than 45% of the freshwater used (H. Liu et al., 2019). Water is a critical issue for rice production in the future and estimated that by 2045 rice fields will experience water scarcity (Boonwicahi & Shrestha, 2018). Planting rice with an irrigated rice field system requires a lot of water from seeding, planting, inundation, and irrigation. With an irrigated rice field system, rice crops water consumption in Turkey is estimated to be between 810-1625 mm. It is also claimed that it takes about 1000-1200 liters of water to produce 1 kg of rice, but this amounts to 4000-5000 liters (Özgenç & Erdoğan, 1988). The difference in water requirements during the growth of rice crops under aerobic and anaerobic conditions is shown in Table 1.

Table 1. Comparison of The Amount of Water Required to Produce 1 Kg of Rice Under Anaerobic and Aerobic Conditions

Water Use Parameters	Water Used During The Rice-Growing Season (mm)	
	Anaerobic Conditions	Aerobic Condition
Soil Preparation	150-300	100
Evaporation	200	100
Transpiration	400	400
Loss of water due to leaks	500-1500	335
Water application efficiency	990-1800	335
Water Use During Rice Planting Season	1650-3000	935

Source : (Tuong & Bouman, 2003)

The most important inhibiting factor in irrigated rice farming is the provision and management of irrigation water. Therefore, it is necessary to develop technology to overcome the scarcity of water. Water management in rice production technologies such as intermittent irrigation, saturated soil cultivation, and intensive drip irrigation can be a future solution. In contrast, aerobic rice cultivation techniques are production techniques developed to address future water shortages with less water, with rice varieties better in water-unsaturated soils such as wheat and maize.

Aerobic rice fields were first developed in China as part of International Rice Research Institue (IRRI) research. Planting rice aerobically can save water regularly by eliminating the need for water at the soil preparation and irrigation stage (Awan et al., 2015). Aerobic rice only requires 800-1000 mm of water, while lowland rice requires more water from 1200 to 1500 mm. Aerobic rice production techniques do not require standing water in the field. The irrigation method used is surface methods such as flush irrigation systems, furrow irrigation, or irrigation sprinkles to keep the soil moist, flooded, and saturated.

As in the world, rice production in Turkey will be affected by global warming in the future. Besides, due to current rice cultivation techniques, there is a higher negative effect of greenhouse gasses than other crops. For example, in 2007, gas emissions from rice cultivation were only 137 kilotonnes of CO_2, increasing 21.4% in 10 years and reaching 167 kilotonnes of CO_2 in 2017 (Tokay, 2018). It is stated that the increase in the earth's temperature will continue to increase from 2.5 – 4^0 C at the end of this century (Dellal & Unuvar, 2019). Increasing temperatures in the world will cause rainfall and soil moisture to decrease and sea-level rise due to climate change.

Referring to changes in agricultural land use in Turkey, there were about 6.6 million hectares of agricultural land in 1928. This figure continued to increase to 25.3 million hectares in 1950 and had reached about 28 million hectares by the end of the 1980s. However, currently, agricultural land has been reduced to around 24 million hectares, and only about 20% is irrigated. The remaining 80% of agricultural land in Turkey depends only on rainfall. Although only 20% of agricultural land is irrigated, more than 70% of existing water resources are used for Turkey's irrigation. We need to make efficient use of water resources to reduce global warming in recent years. Therefore, research on aerobic rice cultivation in Turkey is important to solve this problem in the future.

1. PRODUCTION, CONSUMPTION, AND IMPORT OF RICE IN TURKEY

Commercial rice cultivation in Turkey started 30 to 40 years ago but is relatively new compared to other rice-producing countries. Nearly 90% of rice cultivation in Turkey is carried out in the Marmara and Black Sea Regions, as this region has climatic conditions suitable for rice cultivation. In the last 5 years (2015-2019), the area of rice cultivation area is approximately 110-125 thousand hectares with a growth of 2.04%, rice production has reached 610 thousand tons with a growth of 2.27%, and the average productivity per year is around 7.96 tons/ha which have decreased -0.21% (Table 2). The province with the highest rice production was Çanakkale

Province with 8.44 tonnes/ha. Edirne Province was ranked second with a yield of 8.42 tonnes/ha, where the two provinces were located in the Marmara Region.

Turkey's domestic rice consumption has increased by 1.06% over the last 5 years, and in 2019 rice consumption in Turkey reached 817 thousand tons (Table 2). Annual per capita consumption increased dramatically from about 5 kg before 1990 to 9 kg in 2000 and increased to 16 kg in 2018 (FAOSTAT, 2021). Although Turkey has great potential for rice production due to its suitable climatic characteristics, rice production has not yet reached a good level, resulting in an imbalance between rice production and consumption.

Table 2. Production, Consumption, and Import of Rice in Turkey (2015-2019)

Variable/Year	2015	2016	2017	2018	2019	Growth Rate (%)
Area Harvested (1000 Ha)[1]	116	116	110	120	126	2.04
Yields (ton/ha)[1]	7.94	7.93	8.21	7.82	7.91	-0.21
Production (1000 ton)[2]	552	552	540	564	610	2.27
Domestic Consumption (1000 ton)[2]	783	791	800	808	817	1.06
Import (1000 ton)[2]	245	220	287	253	484	17.16
Import Value (1000 USD)[3]	166	116	149	145	245	11.39

Source : TÜİK (2021)[1]; (Index Mundi, 2021a; 2021b; 2021c)[2]; FAOSTAT (2021)[3]

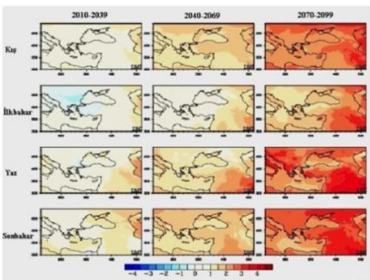
Therefore, the need for rice in Turkey is met through imports. Turkey has been importing rice from Egypt, Italy, and America for the last 5 years. In 2019, Turkey's rice imports from various countries reached 484 thousand tons with a very significant growth of 17.16% (Table 2). The value of Turkish rice imports is directly proportional to the amount of incoming rice, and the value reached 245 million USD in 2019. This value almost doubled the value of the previous imports in 2015-2018, with growth reaching 11.39% (Table 2).

2. REASON WHY SHOULD RESEARCH THE AEROBIC RICE SYSTEM IN TURKEY

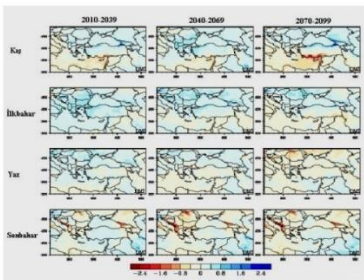
In its natural habitat, rice lives in wetlands and swamps because it is part of semi-aquatic descent, so rice is susceptible to water shortages. Thus, it can be said that the rice crops is the largest water consumer in the world. So it is necessary to make efforts to use water effectively and efficiently to maximize the quality and yield of rice. In aerobic rice production, high yields are obtained from the selection and development of varieties (growth, development, yield, and quality, etc.) from the results of studies conducted under aerobic conditions for many years in terms of anatomical, morphological, physiological, adaptation, and cultivation techniques. Due to global warming and reduced water resources, several countries have researched aerobic rice, which means that similar research is needed in Turkey for the following reasons.

2.1. Impacts of Climate Change

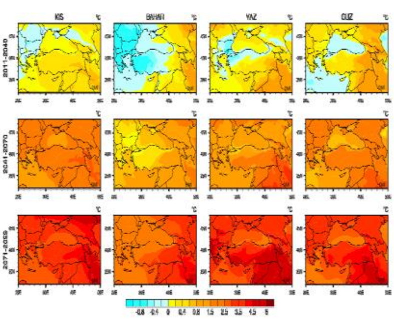
Climate change has been an agricultural research issue in Turkey since 2000. The impacts of climate change have a significant impact on the increase in temperature and the decrease in rainfall to have a significant impact on agricultural production (Akyüz & Atis, 2016; Dellal et al., 2011; Dogan & Kan, 2018; Dumrul & Kilicaslan, 2017; Kanat & Keskin, 2018; Kanber et al., 2008; Türkeş & Erlat, 2006).



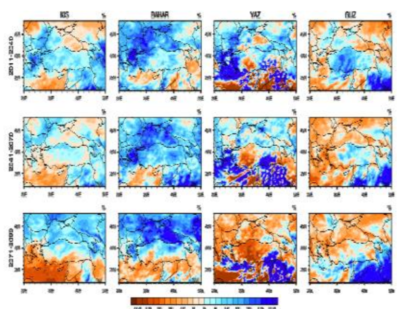
(a) Temperature for scenario B1



(a) Precipitation for scenario B1



(a) Temperature for scenario A2



(b) Precipitation for scenario A2

Figure 1. Projection of Temperature and Precipitation is Based on The Global Climate Model ECHAM5 With The Regional Climate Model Regcm3 For Scenario (A) B1 and B (A2) İn Turkey (MoFAL, 2018a, 2018b).

Climate change and agricultural production have long-term relationships. Climate factors such as CO_2 emissions, temperature changes, and reduced rainfall will have a short, medium, and long-term impact on agricultural activities and grain yields (Chandio et al., 2020). The Turkish State Meteorological Service (MGM, 2020) shows that in 2020 there will be an increase in temperature reaching 1.5^0 C in all regions of Turkey, and an increase in temperature is expected to reach 30^0 C in 2050 and 50^0 C in 2080 (Fig. 1). The increase in temperature results in a decrease in rainfall throughout Turkey, estimated to reach -2 mm/day in 2050 and -5 mm/day in 2080 (Fig. 1) (MoFAL, 2018a, 2018b). An increase in temperature and a decreased rainfall will affect physiological processes and harm rice production (Prasad et al., 2006; Xu et al., 2012).

Table 3. Different Impacts of Climate Change on Rice Production in Several Country

Location	Year	Temperat-ure	Rainfall	Rice Production	Method	Reference
Southern China	1980-2012	Increased 75%	Decreased 64.2%	Decreased 29%-40%	Simulation	S. L. Liu et al., 2016
Africa	2000-2070	Increased 55%-65%	Decreased 22%-43%	Decreased 24%-45%	Simulation	Van Oort & Zwart, 2017
Pakistan	1980-2013	Increased 5%-15%	Decreased 8%-11.5%	Decreased 0.3%-8%	Simulation	Shakoor et al., 2015
Iran	2015-2044	Increased 8%-12%	Decreased 11%-13 %	Decreased 2.1 %-9.5%	Simulation	Gohari et al., 2013
Turkey	2020-2080	Increased 17%-26%	Decreased 20%-32%	Decreased 7%-20%	Simulation	I. Dellal & Unuvar, 2019
Turkey	1990-2100	Increased 19%-29%	Decreased 15%-28%	Decreased 7.3%-12.5%	Simulation	Bozoglu et al., 2019

Table 3 shows that in several countries, including Turkey, climate change has a direct impact on the temperature and rainfall changes. According to Rosenzweig & Iglesias (2006) research, Turkey's agricultural productivity will decrease by 12% in 2080. When observing the parameters of plant water consumption, transpiration, and evapotranspiration in rice crops, I. Dellal & Unuvar (2019) have shown that there will be a decrease in rice production by 7% in 2050 and 13% in 2080 due to climate change.

2.2. Current Conditions of Irrigation and Water Management in Turkey

Turkey is one of the most water-rich countries in the Mediterranean region. However, due to the effects of drought and climate change, Turkey's overall water demand increases. Studies show that Turkey has the highest water security threat level among European countries. Today, Turkey's water resources availability has decreased from about 4000 m^3 to 1500 m^3 per capita/year. This is due to a considerable increase in population from 28 million to 68 million over the last 4 decades. Water availability is expected to continue to decline to 1000 m^3 in 2050 due to population growth and the impact of climate change (Aktaş, 2014).

Approximately 74% of Turkey's total water supply is used for agricultural irrigation. The remaining 15% and 11% are used for domestic drinking and industrial purposes, respectively (Aktaş, 2014; Dogdu & Sagnak, 2008). We can see that the agricultural sector is the largest water user in Turkey, and most of Turkey is experiencing drought during the summer due to water scarcity (Topcu et al., 2019). Nearly 20% of surface water in the Turkish basin will decrease by 2030. By 2050 and 2100, this percentage will increase to 35% and more than 50%, respectively (Ozkul, 2009). By 2100, Turkey could experience an expansion of dry areas, leading to increased water pressure around the southern Mediterranean region (Gao & Giorgi, 2008). The problem of increasing water pressure will have a serious impact on domestic, industrial, and especially agricultural water users.

Currently, of the total agricultural land in Turkey (24 million hectares), only 19% have irrigation water. There is a potential for approximately 60% of surface water sources and 27% of groundwater that are not used. Due to climate change and other technical factors, Turkey's water resources are currently only around 112 km^3/year. This situation still seems to be far from meeting the water needs of agriculture, and there will be water scarcity and salinity of water resources in the future.

In 2010-2019, the Turkish irrigation system only irrigated about 60%-65.8% of agricultural land. The most irrigated crops are cotton, maize, cereals, fruits, and vegetables (Table 4). Approximately 75-85 % of all irrigated areas in Turkey use surface water, and the remaining 20-25% use groundwater (Topcu et al., 2019). Total irrigation water for agricultural irrigation in rice cultivation in Turkey ranges from 788-4355 mm (Ayday & Güngör, 1981; Cakir et al., 1998; Özkara, 1981). Aryal (2012) found in his research that rice crops total water use was 1,261 mm, consisting of 711 mm of irrigation water and 550 mm of rainwater.

Table 4. Irrigated Areas and Plant Distribution in Irrigated Area (2010-2019)

Year	Irrigation Quantity	Area Opened to Irrigation (ha)	Irrigated Area (ha)	Total Irrigation Rate (%)	Plant Distribution in Irrigated Area (%)							
					Cotton	Corn	Sugar Beet	Forage Crops	Citrus	Cereal	Fruit and Vegetable	Others
2010	733	2,213,258	1,393,946	63.0	12.8	22.2	5.9	4.6	3.4	18.5	11.6	21.0
2011	755	2,243,241	1,416,480	63.0	18.9	21.6	5.1	4.4	3.5	14.0	11.8	20.7
2012	774	2,269,542	1,386,954	61.1	15.7	23.5	4.9	5.1	3.4	14.5	11.8	21.1
2013	795	2,256,521	1,428,155	63.3	13.5	25.4	5.0	5.9	3.3	13.2	11.7	22.1
2014	793	2,334,270	1,433,226	61.4	15.1	22.4	4.6	5.9	3.6	14.8	12.3	21.3
2015	833	2,403,753	1,461,444	60.8	13.0	26.0	4.3	5.9	3.6	13.5	12.2	21.5
2016	888	2,428,264	1,577,095	64.9	14.2	4.8	13.4	24.2	6.0	3.2	12.1	22.1
2017	813	2,519,330	1,616,676	64.2	16.7	22.3	5.0	6.1	3.4	12.8	11.5	22.2
2018	968	2,606,280	1,601,967	61.5	19.7	21.9	3.8	6.1	3.4	12.0	11.0	22.1
2019	1,101	2,696,225	1,738,777	64.5	19.4	22.9	4.0	6.9	4.0	9.5	11.8	21.5

Source : DSİ (2020)

Delibaş et al., (2010) calculated the irrigation water requirement of 26,830 m^3 ha^{-1} to irrigate 50,000 hectares of rice fields with a water level of 10-20 cm in the Thrace region. They explained that to obtain that much water supply, the flow rate of the Meriç River is about 1.4×109 m^3, which is the main source of water, but the water flow rate drops to 0.3×109 m^3 during periods when irrigation water is very much needed. The increasing water scarcity during the rice production season threatened the irrigated rice system's productivity and sustainability in Turkey. In the future, it will be important to increase rice production through research and development of rice-growing techniques using the least amount of water to ensure world food demand.

2.3. Weeds Existence and Management

Weed control is one of the most important problems for aerobic rice cultivation. Compared to rice crops in the aerobic rice system, weeds absorb more light, water, nutrients, carbon dioxide (CO_2), grow faster, and cause loss of yield. Various research results show that weeds in aerobic rice cultivation systems can reduce grain yields by 20-100% (Dass et al., 2017; Jabran & Chauhan, 2015; Saravanane et al., 2016; Singh et al., 2018).

The application of weed control and integrated weed management practices in the aerobic rice cultivation system reported an increase in yield of 15-37%. For this reason, it is important to know the types of weeds to determine how weeds can be managed because weed germination and growth are faster in aerobic rice systems than in irrigated lowland rice. Weed species, including *Echinochloa crus-Galli* (L.) P. Beauv., *Leptochloa chinensis* (L.) Nees, *Ischaemum rugosum* Salisb., *Cyperus difformis* L., *Sagittaria montevidensis* Cham. & Schltdl., *Lindernia* spp., *Commelina diffusa* Burm.f., and *Cyperus rotundus* L., were found to have a higher prevalence in aerobic rice systems than irrigated lowland rice systems grown in various countries (Farooq et al., 2011).

Farmers have widely reported that the weeds *Echinochloa oryzoides* are increasing in the main rice-growing regions of Turkey. In Turkey, this species is increasing and becoming increasingly difficult to control (Mennan et al., 2012). This is due to the increasing use of herbicides in such a way that these species experience resistance. This type has very high competitiveness against rice crops in using resources to reduce rice production by up to 40% under conditions of high weed density (Kaya-Altop et al., 2019). In near aerobic conditions, this species can reduce plant dry weight by 17%, indicating that weed competition will increase if the water level is closer to aerobic conditions (Kaya-Altop et al., 2019).

Weed species diversity in aerobic rice cultivation systems needs to be well addressed and managed. Aerobic rice weed management requires integrating different weed management strategies, such as cultural, physical, and biological weed management strategies, and the wise use of herbicides as a last resort rather than the only option.

3. AVAILABILITY OF SUITABLE VARIETIES

The rice varieties which have been grown by farmers in Turkey are not suitable for cultivation aerobic conditions. Therefore, it is necessary to develop rice varieties suitable for aerobic rice ecosystems. Some of the criteria required for aerobic rice varieties are varieties to grow roots in aerobic conditions and grow leaves in aerobic conditions.

3.1. Root Growth Under Aerobic Conditions

Under aerobic conditions, the growth and development of rice varieties have given different responses genetically and physiologically. Experiments conducted by Luiz dos Santos et al. (2018) by providing a water potential threshold of -0.04 MPa (control) to -0.19 Mpa can reduce leaf water potential, water use efficiency, leaf area, and root biomass at the water potential threshold of −0.046 and −0.056 Mpa. Decreased water potential threshold has limited the ability of the rice roots to absorb water under aerobic conditions. Therefore, it is necessary to develop varieties that have a high absorption of water. The roots of plants play an important role in the absorption and transfer of water and nutrients. A dynamic root system will allow plants to obtain more water, and improved root system architecture will prevent plants from drying out under aerobic conditions.

The root system mechanism for water absorption consists of hydraulic conductivity and root hydration, which is affected by the roots total length and roots surface area. Adjustment of root properties includes regulation of the cell membrane's hydraulic conductivity and changes in anatomical root tissue (Tardieu et al., 2017). Adjustment of the roots indicates a change in the root system's structure, as evidenced by the extension of adventitious roots and branching of the lateral roots. This change is caused by a decrease in soil moisture and the absorption of roots into water, which will have a long-term effect on root growth and reduce root biomass.

The condition of changes in the root tissue anatomy is adapted to the conditions of the soil water. Other root anatomy changes, namely cell wall thickening, exodermis lignification, endodermis, and sclerenchyma, have been observed under aerobic conditions (Mostajeran & Rahimi, 2008). Cell walls of rice roots are susceptible to changes in soil moisture conditions so that, under aerobic soil conditions, rice roots develop into aerenchyma (gas-containing air spaces), and the size of the aerenchyma will be affected by the availability of water in the soil (Yamauchi et al., 2016). From the observations of anatomy, morphology, and physiology of the roots, it can be seen that the root system plays an important role in maintaining water scarcity to conclude that root length and root dry weight are key parameters for aerobic adaptation.

3.2. Leaf Growth Under Aerobic Conditions

Water scarcity in rice crops directly affects rice leaves anatomical, morphological, and physiological functions. In aerobic conditions, symptoms of drought are caused by reduced soil moisture and water absorption by rice. Anatomical changes in rice leaves are indicated by the characteristics of stomatal density, stomatal size, mesophyll conductivity, and mesophyll thickness. Changes in the morphology of rice leaves cause rice leaves to roll and the aging rice leaves. Physiological changes are observed with changes in chlorophyll content, proline, transpiration rate, and photosynthetic rate.

Photosynthesis is the main process in primary metabolism and directly affects rice productivity (Gago et al., 2016). In an aerobic situation, photosynthesis will be affected by the rubisco rate and limited by the CO_2 concentration in chloroplasts carbolaction. This process is determined by the diffusion component of CO_2, namely the stomata's conductivity and the mesophyll's conductivity (Evans et al., 2009).

In water scarcity conditions, plants close the stomata to prevent large water losses that lead to CO_2 absorption (Pinheiro & Chaves, 2011). In the long-term response, under conditions of water scarcity, the stomata's conductivity may affect the density and size of the stomata (Yang et al., 2020). The stomata in the leaves control the diffusion of CO_2 to the plant leaves and the diffusion of water through the plant leaves.

The result of the entry of CO_2 from leaf stomata is water loss through transpiration, thus affecting the efficiency of photosynthesis and transpiration rates (Li et al., 2017). Meanwhile, the conductivity process occurs in the mesophyll of the chloroplast. This process regulates the diffusion of CO_2 from the sub-stomatal space to the carboxylation site, thereby increasing the rate of photosynthesis without increasing transpiration. This is because the CO_2 diffusion pathway involving the stomata's conductivity is not divided into the

H_2O diffusion pathway, which is transpired (Flexas et al., 2015). Therefore, investigating the relationship between anatomical, morphological, and physiological functions with photosynthetic properties in rice leaves can improve understanding of rice leaves structural properties needed to increase their tolerance to aerobic conditions.

4. RESEARCH OPPORTUNITIES FOR THE DEVELOPMENT OF THE AEROBIC RICE SYSTEM IN TURKEY

Looking at Turkey's ecological conditions, the aerobic rice system is one technology that is worth considering in Turkey. Given the many challenges ahead in rice production activities, such as climate change and scarcity of water in meeting the needs of lowland rice irrigation. Whereas in recent years, drip irrigated rice cultivation in the Bafra Plain (Karadeniz region) has been carried out, but this opportunity has not been optimal.

Further research needs to focus on finding aerobic rice varieties that are drought tolerant with high yield characteristics. Aerobic rice only requires less water intake (50%) and higher water productivity (66-88%) compared to the lowland rice system (Sharma et al., 2016). Finally, this rice variety must be associated with low inputs, drought-sensitive, pest, and disease resistance (Dar et al., 2020).

Currently, Turkish researchers have conducted several studies on the development of drought-resistant rice varieties. Beser et al. (2015) reported the selection of 22 Turkish rice varieties, and 3 varieties were able to produce high in drought conditions. The Durağan, Osmancık-97, and Halilbey rice varieties can be adapted to drip irrigation techniques and grown under conditions that use about 50% less water than irrigated rice systems. The three varieties yields were 229.38-651.71 kg ha^{-1}, the harvest index value was 21.30%-47.50%, the weight of 1000 seeds was 20.74-35.99 gr. The results of research conducted by Tuna (2012) show that the Osmancık-97 variety can be adapted to dry conditions with water availability of 723-1446 mm with drip irrigation. Kuru (2017) reports that the Osmancık-97 variety has a lower H_2O_2 content, higher antioxidant enzyme activity, and a higher amount of ABA, which indicates that the stress hormones of this variety are more resistant to drought.

Further research on aerobic rice cultivation techniques is needed to find rice varieties weed competitiveness and extensive studies on rice weed ecology for weed control. As explained above, weeds are a major obstacle to aerobic rice production. The development of rice varieties weed competitiveness will reduce environmental pollution and reduce the use of herbicides. Weed competitive rice varieties are reported as low-cost and safe tools for integrated weed management (Bahadur et al., 2015). Turkish researchers have not conducted studies testing the resistance of rice to weed attack under aerobic conditions. Therefore, different weed management strategies need to be integrated, such as cultural, physical, and biological weed management strategies, to ensure effective and sustainable weed management in aerobic paddy fields.

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Small farmers produce more than 90% of rice production globally and in Turkey, and rice production in Turkey is produced from 95% of irrigated rice fields under anaerobic conditions. Under current conditions, the main problems of sustainable irrigated lowland rice production are limited by water scarcity, inadequate irrigation systems, water pollution, and competition with other crops. Seeing these problems, Turkey's aerobic rice cultivation system is very feasible, as are several other countries in the world. Aerobic rice cultivation systems will significantly reduce future climate change by reducing greenhouse gas (methane) emissions.

The main point in the development of aerobic rice varieties is the relationship between the anatomical structure, morphology (development of roots, leaves, etc.), and the photosynthetic properties of rice under aerobic conditions. Furthermore, aerobic rice varieties development must be carried out by discovering the genetic potential of local Turkish rice, suitable for farmers cultures and environmental conditions. Therefore, aerobic rice varieties development must refer to the socio-ecology of rice crops in Turkey, which are located in the Marmara and Black Sea regions (about 80%) and other areas to increase the area of aerobic rice cultivation.

Following the development of the varieties, the next step is to prepare an aerobic rice cultivation technique package. These include soil processing techniques, seedbed preparation, planting time, planting depth, irrigation, fertilization, weed control, diseases, and pests. Adjustment of the aerobic rice cultivation technique package optimally will increase the amount of aerobic rice production economically. Support for farmers who use aerobic rice cultivation techniques should be provided from both the public and private sectors. So that in the future, aerobic rice cultivation techniques in Turkey can be adaptable and high yield-oriented to achieve efficient.

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CHAPTER 9

EDIBLE FLOWERS AS EDIBLE ORNAMENTALS:

A REVIEW

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INTRODUCTION

For millennia, phytotherapy has employed herb blossoms. Since ancient times, fresh flowers have mostly been utilized to beautify and scent the homes. A new trend that has recently been noticed involves the widespread ingestion of mostly fresh flowers. In addition to their widespread use as ornaments, flowers are believed to be consumed as food in many different civilizations around the world as a component of traditional cuisine or alternative medicine. Gourmet cuisine uses edible flowers more frequently because they add a delicate flavor, a fresh and exotic aroma, and a beautiful appearance.

The market now offers realistic and safer alternatives due to shifting eating patterns and the search for better living (Pires et al., 2019). People all throughout the world are requesting cuisine that is more enticing and delicious. The aesthetics of ingested meals are influenced by both the food's quality and other factors. Edible flowers could increase the allure and charm of some foods. Fresh, premium flowers for human consumption are becoming more and more popular in today's society. These items, which are properly packaged in bunches, boxes, etc., are offered for sale either directly in farm shops or through a variety of specialty stores (Mlcek & Rop, 2011).

In countries like the UK, Portugal, and Australia, edible flowers have become the newest food trend, making this specialist industry extremely difficult to penetrate. Around the world, there are more producers and outlets for edible flowers (supermarkets, neighborhood markets, internet), but customers and chefs desire higher-quality goods (Fernandes et al., 2020). For millennia, phytotherapy has employed herb blossoms. Since ancient times, fresh flowers have mostly been utilized to beautify and scent the homes. A new trend that has recently been noticed involves the widespread ingestion of mostly fresh flowers. At addition to being a popular topic for magazine stories, edible flowers are frequently offered in chic restaurants, providing an excellent chance for illustrations (Matyjaszczyk & Miechowska, 2019).

1. EDIBLE FLOWERS IN HISTROY

In China and Japan, edible flowers have been consumed for thousands of years (Rop et al., 2012). Ancient Greece and Rome employed a variety of edible flowers as relishes and flavor enhancers for a variety of sweet and savory foods. For instance, in ancient Rome, several kinds of purées and/or omelets were prepared using the blossoms of different species of roses (Rosa spp.). The blossoms of the calendula plant (Calendula officinalis) were used to make a variety of salads in medieval France. Similarly to saffron (Crocus) inflorescences, this species' flowers are still employed as food coloring ingredients. Violets (Viola odorata) were used to color sugar, syrups, and different remedies in the 17th century. Flowers from dandelion (Taraxacum officinale) plants were used to make beverages and salads throughout Europe. Inflorescences of the fried elder (Sambucus nigra) were popular foods in Central Europe (Mlcek & Rop, 2011).

In Nepal, there are many different castes and ethnic groups, and the indigenous people—the majority of whom reside in rural areas—rely on conventional medical procedures. One of the 125 castes and ethnic groups of Nepal is the Kisan, which is only found in the Jhapa district. The Kisan community traditionally uses 40 types of medicinal plants from 32 families to treat 34 different ailments. Traditional knowledge has been linked to Kisan religious beliefs and practices. Only old people and a small number of local traditional healers have access to this knowledge. The indigenous Kisan people have a thorough understanding of the medicinal plants in their area, which also reflects their spiritual traditions and worldview. Flowers of Camellia sinensis (L.) Kuntze is consumed by them as tea plant and Chiya (consumed as vegetables) (Rajbanshi & Thapa, 2019).

Fig. 1. Some Mexican edible flowers, a) Yucca, b) Coral tree, c) Mexican marigold, d) cactus, e) Plumeria, f) Squash, g) Funeral tree, h) Dahlia, i) Agave, j) Poinsettia (Photos: Ray Rodriguez) (Mulik & Ozuna, 2020)

In Malaysia, "Nasi Kerabu," a rice-based dish, is colored with Clitoria ternatea (blue pea) flower petals, while "Penang Rojak," a famous salad dish, is made with torch ginger flower (Takahashi et Lal., 2020). The ethnic people of northern Thailand regularly uses edible flowers including Bougainvillea glabra, Tagetes erecta, Cosmos sulphureus, and Antigonon leptopus to make salads and flower teas (Kaisoon et al., 2012). Agave salmiana, Aloe vera, Arbutus xalapensis, Cucurbita pepo (cultivated), Erythrina americana, Erythrina caribaea, Euphorbia radians benth, and Yucca filifera are among the edible flowers found in Mexico (Sotelo et al., 2007).

The anti-inflammatory, hepatoprotective, neuroprotective, anti-cancer, anti-diabetic, anti-osteoporosis, anti-obesity, and anti-hypertensive properties of floral extracts have been studied, and their effective doses have been compiled. Before 2015, the terms "anti-osteoporosis," "anti-obesity," and "anti-hypertension" were hardly ever used in connection with health benefits. After 2015, a few health advantages of eating flowers were regularly addressed. It has been demonstrated that some recently discovered phytochemicals, such polysaccharides, are good for human health. There have been reports of certain rosa, chrysanthemum, and osmanthus species having an impact on human health. The safe level of floral extracts in cell and animal models for the toxicity tests was at a level of hundreds of parts per million (ppm). In consideration of health promoting effects and toxicities of edible flowers, they could serve as potential natural health products for different health benefits (Zheng et al., 2021).

While they are employed in food preparation and improve the aesthetic appeal of meals, edible flowers are more frequently referenced in relation to biologically active compounds (Mlcek & Rop, 2011).

2. CURRENT TIMES

In order to popularize and grow this expanding sector, socio-cultural elements related to the consumption of edible flowers have been the focus of numerous studies. Encouraging local use of traditional flowers is crucial to maintain endangered traditions. Contrarily, with the increased interest in natural and healthy foods, the nutritional qualities, pharmacological advantages, chemical makeup, and methods of preparation of edible species have all been explored more and more (Takahashi et al., 2020).

Different species of edible flowers have different colours and taste: Centaurea cyanus (blue, vegetal flavour), Antirrhinum majus (yellow, bitter flavour), Chrysanthemum frutescens (orange yellow, slightly to very bitter flavour), Fuchsia x hybrida (reddish and pinkish purple, slightly acidic flavour), Dianthus caryophyllus (dark pink, slightly bitter flavour), Impatiens walleriana (pink, sweet flavour), Rosa odorata (red, sweet and aromatic flavour), Viola x wittrockiana (two coloured petals—yellow and violet, sweet flavour), Tropaeolum majus (red, sharp and cress-like flavour), Chrysanthemum parthenium (white yellow, slightly to very bitter flavour), Begonia boliviensis (reddish orange, slightly lemon flavour), Tagetes patula (orange, bitterish, clove-like flavour). Non poisonous edible flowers are described here. However, it should be noted that the maximum daily intake for eating flowers from ornamental plants is not yet established (Mlcek & Rop, 2011).

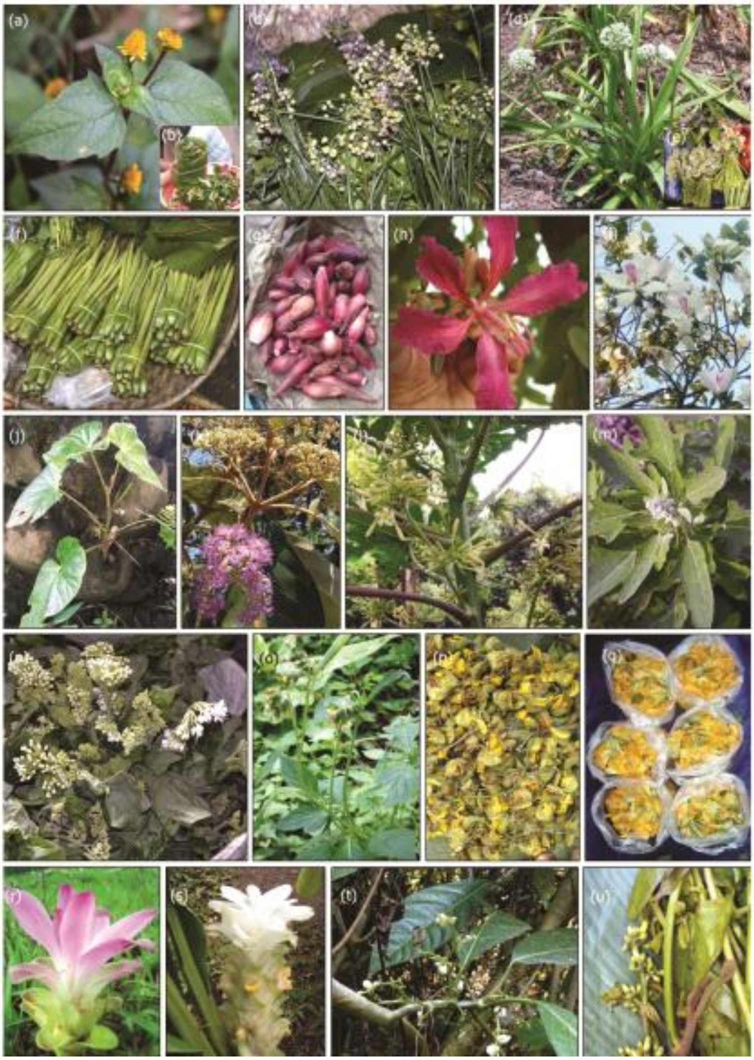

Fig. 2. Edible flowers (inflorescence) of Mizoram: – (a and b) Acmella paniculata; (c) Allium chinense; (d and e) Allium hookerii Thwaites; (f) Alocasia fornicata; (g) Amomum dealbatum Roxb.; (h) Bauhinia purpurea L.; (i) B. variegata L.; (j) Begonia longifolia Blume; (k) Callicarpa arborea; (l) Carica papaya; (m) Chenopodium album L.; (n) Clerodendrum glandulosum Lindl.; (o) Crassocephalum crepidioides (Benth.) S. Moore; (p) Crotalaria tetragona Andrews; (q) Cucurbita maxima Duchesne; (r) Curcuma angustifolia; (s) C. longa; (t) Dendrocnide sinuata; (u) Dysoxylum excelsum Blume (Khomdram et al., 2019)

In literatures, some species have been deeper evaluated such as lavender (Lavandula pedunculata Cav.) (Nowicka & Wojdyło, 2019), hibiscus (Hibiscus rosa-sinensis L.) (Antarkar et al., 2019), pansy (Viola ×

wittrockiana Gams) (Fernandes et al., 2019c), peony (Paeonia suffruticosa Andr.) (Fernandes et al., 2019a) etc. Additional other edible flower producing plants are given here below with their taste taken from publishment of Newman & O'Connor, (2009):

- **Abelmoschus aesculentus** (Okra, gumbo, gombo) / Mild, sweet and slightly mucilaginous

- **Agastache foeniculum** (Anise hyssop) / Strong anise, sweet, licorice

- **Alcea rosea** (Hollyhock) / Little, slightly bitter

- **Allium schoenoprasum** (Chive) / Onion, strong

- **Allium tuberosum** (Garlic chive) / Onion, strong

- **Anethum graveolens** (Dill) / Stronger than leaves

- **Anthemis nobilis** (English chamomile) / Sweet apple flavor

- **Anthriscus cerefolium** (Chervil) / Parsley-like, hint of citrus, tarragon

- **Begonia x tuberhybrida** (Tuberous begonia) / Citrus

- **Bellis perennis** (English daisy) / Mild to bitter

- **Borago officinalis** (Borage) / Cucumber

- **Brassica spp.** (Broccoli, cauliflower) / Broccoli

- **Brassica spp.** (Mustard) / Mustard, hot

- **Calendula officinalis** (Calendula, pot marigold) / Tangy and peppery

- **Carthamus tinctorius** (Safflower, American safflower, saffron) / Bitter flavor

- **Centaurea cyanus** / Mild

- **Cercis canadensis** (Redbud) / Beanlike to tart apple.

Consumers judge flowers based on their appealing size, shape, flavor, aroma, and color. Flowers with these colors are more popular among consumers. One of the most crucial qualities of edible flowers is aroma. For a particular species of flowers, odor is frequently a defining characteristic. Flowers were the subject of sensory evaluation experiments by Benvenuti et al. (2016). Spices, sweetness, scent, suppleness, and bitterness were among the characteristics that customers evaluated. The consumers decidedly preferred A. houstorianum, B. semperflorens, V. vittrockiana i T. majus due to their tastiness.

Dahlia mignon, Rosa damascena ‘Alexandria’ and R. gallica ‘Francesa’ draft in R. canina, Calendula officinalis L., and Centaurea cyanus L. are among the most popular edible flowers (Fernandes et al., 2017).

The phenolic components and antioxidant properties of free and bound phenolics from 12 Thai edible flowers were studied by Kaisoon et al. in 2011. These flowers have long been consumed as vegetables and used as ingredients in recipes. The highest value for total phenolic content (TPC) was found in Cassia siamea (88 mg gallic acid equivalents (GAE)/g dry weight). The highest total flavonoid concentration (TFC) was found in Tagetes erecta (68.9 mg RE/g dry weight). Leptopus Antigonon and T. The ferric reducing antioxidant power (FRAP) value for erecta was greatest (62.0 and 60 mmolFeSO4/g 100 dry weight). Gallic, ferulic, and sinapic acids were the three major phenolic acids found in these studies, whereas quercetin and rutin were the two most common flavonoids. According to the study's findings, both the soluble and bound portions of edible flowers are abundant sources of phenolic compounds that have reducing, antioxidant, and DPPH radical-scavenging properties.

4. CONSUMPTION OF EDIBLE FLOWERS

Edible flowers can be consumed fresh (eg. marigold flowers in salads), as well as in savory dishes containing meat and fish, in soups and drinks (wine, beer), in desserts, sweets, jellies, as well as spices, and dyes. They are used in dry form (infusions, dried rose petals in desserts), as powder (Chen & Wei, 2017), crystallized or as foams (molecular gastronomy) (Fernandes et al., 2019).

It is crucial to correctly identify the plant because some blossoms are dangerous and shouldn't be eaten. Numerous plants share similar common names, which could further muddy the waters. Always choose flowers based on their scientific names. Pick flowers in the morning. For the finest flavor, use them when they are fresh. Avoid unopened flowers, with the exception of daylilies, as well as wilted or fading blooms since they could taste harsh or unpleasant. Never use pesticide-treated flowers or pick flowers from plants that have received raw manure fertilizer. In general, stay away from buying flowers from florists, garden centers, or nurseries as they are not produced for human use. You can also save fresh flowers for later use. Pick flowers with larger petals, like pansies, and egg-white wash the petals on them. To prevent food-borne illness, use a delicate brush and pasteurized dehydrated egg whites. After painting, dry the petal and then sprinkle it with ultra-fine granulated sugar. In a cool, dark location, keep preserved flowers in an airtight container. Avoid using this procedure on petals with dark colors as they will become even darker. Consumption should be restricted to tiny tastes in order to prevent stomach distress or to identify any adverse reactions. Introduce significant amounts of food into your diet gradually. You can consume flower petals or even the whole flower. However, because they could be bitter, remove the stems, anthers, and pistils. Use flower which are pest- and disease-free. Numerous edible flowers are rich in critical vitamins like vitamin C and/or A. Use them in salads and as garnishes. There are flower recipes for baking, sauces, jelly, syrup, vinegars, honey, oil, tea, candied flowers, wine, and flavored liqueurs, among other things. Homemade flavored oils and vinegars have a short shelf life and should be kept in the fridge. After selecting fresh flowers, gently rinse them under cool running water. Place the washed flowers between damp paper towels. Keep chilled until you're ready to use. If you wash some flowers right before using them, their shelf life may be extended. Drying flowers and plants is another option.(Newman & O'Connor, 2009; Kendall and Rausch, 2012).

5. POST HARVEST TECHNOLOGIES APPLIED TO EDİBLE FLOWERS

Technologies used after harvest to preserve the freshness and safety of edible flowers aid producers in growing their market share and educating consumers about these technologies. New post-harvest techniques including irradiation and high hydrostatic pressure (HHP) have produced successful outcomes. In comparison to traditional drying methods, freezedrying or vacuumdrying have proven to be extremely effective at preserving the bioactive components of flowers. The use of edible coatings and films can be a healthier alternative to osmotic dehydration while without increasing the solute levels, even though it is already in use (Fernandes et al., 2019).

6. EDIBLE LANDSCAPING IN URBAN HORTICULTURE

Edible landscaping is simply a way of using veggies, herbs, fruits, and flowers that will perform multiple functions, such as for food, flavor, and ornamental appearance. Alternatives to traditional landscaping that produce fruits, vegetables, and herbs for domestic use include edible landscaping. Edibles can be planted separately or combined with ornamentals to create aesthetically beautiful designs in already existing yards and gardens. Many edibles function in landscape in just a perfect way, i.e., date palm is extensively used in landscape as a major tree, rosemary that is used as spice is a suitable for low-growing perennial hedge, and purple basil is perfect alternative for dark-leaved annuals for pots (Fetouh, 2018).

Urban agriculture represents an opportunity to improve food supply, health conditions, local economy, social integration, and environmental sustainability (Taheri et al., 2022).

Known for their ornamental appeal, ornamental peppers (Capsicum annuum L.) have a variety of morphological features. For fruit and leaf shape and size, as well as plant habit, Capsicum germplasm exhibits a significant degree of variety. This morphological diversity offers a wide range of chances to create distinctive decorative cultivars, along with a variety of ripe fruit colors and varied shades of green to purple foliar coloring. The popularity of ornamental pepper cultivars is mostly due to their simple seed propagation, quick harvesting times, resistance to heat and drought, and superior preserving qualities. These qualities have made it possible to use ornamental peppers as pot plants, novelty items, cut stems, bedding plants, and garden plants. The availability of disease resistance in other pod types provides opportunities to introduce resistance into ornamental peppers. Renewed interest in ornamental peppers has stimulated new breeding activities. Knowledge gained from genetic studies and breeding of culinary type peppers affords valuable information for use in developing new ornamental pepper cultivars (Stommel & Bosland, 2007).

Helianthus annuus L., the sunflower, is primarily grown as a staple crop for the production of edible oil as well as a source of protein and renewable energy. However, this species' bright and varied blossoms led to one of its earliest uses—as an ornamental plant. Additionally, over the past ten years, the popularity of ornamental sunflowers has grown, which has enabled the introduction of new types for use as cut flowers, pot plants, or garden plants (Mayor et al., 2010).

CONCLUSİONS

The agronomic realities of horticulture and floriculture have always been parallel and extremely different. While horticulture is focused on satisfying food demands, floriculture is concerned with supplying the beauty needs of our urban ecosystems.

Despite being used recently to add flavor, color, and aesthetic appeal to meals, the potential of flowers as human food is still underestimated. Since tons of flowers are thrown away every day, regardless of their nutritional and functional value, because they lose ornamental qualities, the development of products comprising edible ornamental flowers may be a sustainable solution to the cut flower industry.

Flowers with properties that are more than just a matter of flavor may be beneficial to sick people, especially flowers with anti-inflammatory, hepatoprotective, neuroprotective, anti-cancer, anti-diabetic, anti-osteoporosis, anti-obesity, and anti-hypertensive properties.

Although the benefits of flowers as a new, potential source of minerals for human nutrition should not be overlooked, caution must be exercised in light of the occasionally produced anti-nutritional compounds by specific species.

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CHAPTER 10

FLORICULTURE SECTOR CUT FLOWERS MARKET: A REVIEW

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INTRODUCTION

The primary drivers for ornamentals demand are influenced by socio-economic and demographic factors. Domestic and international product flows pivot around the logistic hubs of the large cities in the world. The EU holds the first place in cut flower and ornamental pot-plant sales value in the global, followed by China and USA. Main cut plant species are Roses, Tulips, Chrysanthemums, Gerbera, Freesia, Liliies, Carnations, Alstroemeria, Eustoma russellianum, Hypericum, Gypsophila, Paeonia, Limonium, Zantedeschia, Anthurium, Hippeastrum, Hydrangea, Gladioli, Iris, Snapdragons, Delphinium, Orchids, Lisianthus, Cymbidium, Leatherleaf Ferns and Sunfower.

Here in this review, main cut plant species, key countries, major cut flower wholesalers, key companies are informed and some of them are analysed in details.

Socioeconomic and demographic considerations are the main drivers of the market for ornamentals. For instance, the consumption of non-necessity consumer products, such as ornaments, will start to increase as disposable household earnings rise. Domestic and international product flows revolve around the huge metro clusters' logistical hubs, which are frequently located in the global centres. These hubs have expanded to their current sizes and levels of connectivity for a purpose; they are dominating as a result of their sizable populations and strong economic output. They are home to the target populations that either currently or in the future will be responsible for driving the demand for ornaments worldwide. A central core city with surrounding periphery cities which all together combine into a greater urban area spanning multiple municipalities, counties and sometimes even countries is more than a city. With a GDP of €3.520 billion and 74 million residents, the urbanization zone that mostly runs along Japan's Pacific coast, which includes cities like Tokyo, Yokohama, Nagoya, Kyoto, and Osaka, is ranked as the world's top urban region. The present list of cores will soon include additional sizable cities in developing nations. Growth in relatively isolated demand hotspots in and around their own core cities will be seen in these developing regions of South East Asia, Latin America, and Africa. Due to the lack of international trade, most of the need is currently satisfied by domestic manufacturing. However, as disposable income increases, demand and behavior are likely to shift, increasing the possibility for ornamental imports through external commerce. Countries that have attracted the manufacturing of ornaments primarily because of their natural or economic resources are known as mature exporting producers. For instance, Ecuador and Colombia stand out for having high altitudes that are ideal for growing premium products like huge bud roses. In East Africa, Kenya and Uganda can provide locations at altitude, good climatic conditions and strong workforces. Instead, established domestic companies already meet the vast majority of demand through their own output. They are currently the greatest consumer markets and are all located in the present world cores. With a production area of over 2 million hectares for ornamentals but little external trade, India is another developing domestic producer nation. The need for ornaments, and in particular cut flowers, is enormous and deeply ingrained in culture. The majority of the production base—nearly 90%—is dedicated to the cultivation of so-called loose flowers for religious ceremonies. With only €20 million in imports and €100 million in exports, the external commerce is insignificant (AIHP, 2019c).

With 31.0% of the global value of sales of cut flowers and ornamental pot plants, the EU leads the field, followed by China and the USA with 18.6% and 12.5%, respectively. In the EU, sales of cut flowers and ornamental pot plants peaked in 2016, with the Netherlands leading, followed by France and Italy (Eurostat, 2017).

Between 2007 and 2015, there were significantly fewer growers of cut flowers in the USA. Production of cut flowers has shifted to new global players like China. China ranked second in sales of potted plants and cut flowers in 2017. (Eurostat, 2018).

2. WORLD FLORİCULTURE MARKET

Dummen, Syngenta Flowers Inc., Oserian Development Company Ltd., and Selecta Klemm GmbH & Co. are among the industry's major market participants in the floriculture market. Ruparelia Group, KG. Growing demand for cut flowers during festivals in nations like China and India, together with increasing awareness of these blooms, is projected to support current floriculture market trends. The development of specialized retail outlets like supermarkets in industrialized nations like the UK would increase demand for floriculture products. In 2018, the cut flower market generated close to USD 32.52 billion in revenue. The greatest market share in the global floriculture industry is projected to reside in Europe. Additionally, the market is anticipated to grow between 2022 and 2029 due to the region's increased demand for potted or flowering plants. Europe is the region with the biggest revenue-generating plants and flowers due to the establishment of the Dutch auctions, a significant trade system. On the other hand, North America experiences the fastest increase as a result of the rising popularity of floral decoration and other events. The USDA estimates that floriculture sales were USD 32 billion in 2015 (GlobeNewswire, 2021).

Roses, Chrysanthemums, Tulips, Lilies, Gerberas, Carnations, Texas Bluebells, Freesias, and Hydrangeas are the most popular flowers in the floriculture industry. By end use, the floriculture market is divided into four categories: personal use, institutions/events, hotels, resorts, and spas, and industrial usage. Direct sales of floral products, sales through specialty stores, sales through franchises, sales through florists and kiosks, sales through supermarkets and hypermarkets, sales through independent small stores, and sales through online retailers are the main sales channels for the floral industry (FMI, 2022).

Key Countries are U.S., Canada, Mexico, Brazil, Mexico, Argentina, Germany, Italy, France, U.K, Nordic, Spain, Japan, China, India, Malaysia, Thailand, Australia, South Africa, Turkey. Key Companies are Forest Produce Ltd., Selecta Cut Flowers S.A.U., Native Floral Group, Tropical Foliage Plants Inc., Oserian Group, Esmeralda Farms, Marginpar BV, DOS GRrINGOS LLC, Flamingo Horticulture Ltd., Danziger Group, Florensis Flower Seeds UK Ltd., Verbeek Export B.V., Florance Flora (FMI, 2022).

Among a select group of health-conscious consumers, edible flowers including citrus blossoms, hibiscus, lavender, roses, and nasturtium are becoming more popular. These flowers are abundant in antioxidants and Vitamin C, which boost skin and hair health, reduce cholesterol levels, and improve heart function. For manufacturers in the advanced floriculture sector, expanding their businesses presents new prospects due to the rising popularity of flowers as a nutritious food element. In order to lessen the influence of their operations on the environment, all parties involved in the floriculture value chain strive to embrace sustainable agricultural methods, such as combining pest management and conservation agriculture. The advanced floriculture sector has consumers who are also willing to pay extra for flowers that are farmed sustainably. To strengthen their position in the market, businesses are pursuing certifications and providing fair trade, FPP, and other certified flowers to customers. Due to the growing popularity of gardening activities in the area, Europe is anticipated to maintain its leadership position in the global floriculture market. On the strength of the expanding market for floriculture plants, such as cut flowers and decorative plants, the U.K. and Germany are leading the regional landscape. Furthermore, tulips and chrysanthemums are among the plants used in floriculture that are most commonly imported from the Netherlands. Additionally, improvements in e-Commerce are anticipated to benefit the European floriculture industry. Online growers are becoming more and more well-liked in the United States because they allow consumers to buy flowers at fair costs and of good quality by bringing together producers and purchasers through online auctions. India is quickly becoming a significant producer of plants used in floriculture. The country's commercial floriculture industry is starting to flourish as flower demand rises. Indian culture has always valued flowers, which are grown for a number of purposes, including artistic, cultural, and religious ones. Cut flowers continue to be the primary source of income for market players, with an 80.9% market share. Cut flowers are frequently used in bouquets, wreaths, and vase designs. Despite the fact that cut flowers are also sold commercially, many gardeners cut their own blooms from their floriculture nurseries. The plants that are grown are influenced by the environment, tradition, and local economy. In the field or in a glasshouse, plants are frequently grown specifically for this function. The development of new technologies in the cut flower industry to boost output and utilization would support market expansion overall. Institutions and events that currently account for around 3/5 of the industry would continue to be the principal consumers of floriculture products. Flowers are bought for a variety of purposes, including expressing love or sympathy, as well as achieving environmental and aesthetic aims. In order to add green cover into office interior designs, floriculture is currently being used. In many nations around the world, flowers are used for greeting one another and as decorations at celebrations, which will promote segmental growth. Since proper cold chain management has a beneficial impact on product quality, demand for cold chain protocols is quickly expanding among B2B flower buyers. As a result, businesses concentrate on creating cold chain protocols, which are expected to be essential for long-term growth in the near future. The competitive environment in the worldwide floriculture market is very fragmented, and the majority of the leading companies continue to concentrate on developing plant biotechnology, growing the output of flowering plants, and strengthening their market positions (FMI, 2022).

Key countries in cut flower sector are U.S., Canada, Mexico, Brazil, Mexico, Argentina, Germany, Italy, France, U.K, Nordic, Spain, Japan, China, India, Malaysia, Thailand, Australia, South Africa, Turkey.

Table 2. Flowers and ornamental plants (area, production value and number of enterprises) data of major countries

	Area (ha)				**Production Value**		**Number of Enterprises**	
	In protection	**Open field**	**Total**	**Year**	**Million EUR**	**Year**	**Number**	**Year**
China			184.586	2018	7.739		70.758	2018
US	20.078	8.078	28.155	2017	3.297	2017	39.300	2017
Netherlands	3.920	3.540	7.460	2020	2.379	2019	3.320	2020
Germany	1.703	4.885	6.588	2017	1.391	2019	3.668	2017
Italy	5.443	7.282	12.724	2010	1.269	2019	14.093	2010
Colombia			7.665	2018	1.236	2020		
Japan			17.800					
Spain	2.325	3.896	6.221	2018	961	2019	-	-
France	-	-	6.390	2020	957	2019	3.308	2017
India			313.000					
Ecuador	5.526	931	6.457	2020	741	2020		
Poland	1.525	3.844	5.369	2020	531	2020	-	-
Kenya			4.039		501	2020		
UK	450	6.300	6.750	2020	474	2020	-	-
Ethiopia	-	-	1.695	2017	245	2019		
Portugal	610	1.090	1.700	2010	225	2019	-	-
Costa Rica			3.600	2018	105	2020		
South Africa			11.461		83	2019		
Brazil			15.600	2020			8.300	2020
Thailand			12.324					
Mexico	1.543	8.155	9.698	2020				
Iran	3.338	2.879	6.267	2020				
Australia	314	4.212	4.527	2020			539	2020
Vietnam			4.500					
Israel	1.748	1.000	2.748	2004	-			
Malaysia			2.702					
Korea Republic			2.611					
Turkey	1.390	-	1.390	2020	-			
Others			-					
World			734.000		34.000		300.000	

Key Companies are Selecta Cut Flowers S.A.U., Forest Produce Ltd., Native Floral Group, Oserian Group, Tropical Foliage Plants Inc., Esmeralda Farms, DOS GRrINGOS LLC, Flamingo Horticulture Ltd., Marginpar BV, Danziger Group, Florensis Flower Seeds UK Ltd., Florance Flora and Verbeek Export B.V. (FMI, 2022). Also Kariki, Karen Roses, Multiflora, Harvest Flower, Queens Group, Afriflora, Ball Horticultural, Karuturi Global Ltd., Selecta One, Oserian, Washington Bulb, Carzan Flowers, Arcangeli Giovani & Figlio, Rosebud, Danziger, Sakata, Benary, Oserian and Selecta

One are also key companies. Additionally, advancements in cold storage technology, better supply management, and the use of biotechnology to create distinctive and disease-resistant flower types are anticipated to fuel floriculture market expansion (MMR, 2022).

The demand for the product is rising globally as a result of the rising sales of flowers through both online and offline distribution channels. Valentine's Day is the greatest floral occasion of the year, followed by Christmas and Mother's Day. Birthdays and anniversaries account for 29% of annual sales, whereas 77% of flower purchases are made for a special occasion. The demand for the product is being fueled by the increased application of biotechnology solutions in floriculture to genetically alter the color, shape, postharvest life, abiotic stress, and insect resistance of blooming ornamental plants. Additionally, flowers can live in harsh climatic circumstances thanks to biotechnology methods, which also lessen the dangers for flower growers (MMR, 2022).

The floriculture market is expanding due to the expanding e-commerce sector, as well as the widespread use of smartphones and the internet. In 2021, the Cut Flowers sector accounted for 83% of the market. In 2021, the Gift segment accounted for 57% of the market, which was the highest proportion. The increased usage of flowers as gifts for special occasions or events, such as birthdays, family gatherings, Valentine's Day, Mother's Day, and others, is credited with driving the rise of the floriculture market globally. About 20% of those are bought for homes or businesses, while 20% are bought for weddings or funerals. The Asia Pacific region is expected to hold the highest share in the Floriculture Market. Potted plants account for 15% of the US $ 50.5 Bn floriculture market (MMR, 2022).

Consumers' tastes are evolving, as is their awareness of preferred products. Their purchasing practices for flowers are becoming more refined. There will still be some global trade, but procurement and production will always be local affairs. This global trade will be constrained to particular seasons, particular goods, particular production circumstances, and natural disasters (Anumala & Kumar, 2021).

The field of floriculture has enormous potential and is now a key component of contemporary living. The floriculture sector has evolved into a commercial business with a strong market value and viable opportunities. Around 145 nations are participating in this industry globally, with western nations including the Netherlands, the United States, Columbia, and Italy serving as the top growers and traders. Netherlands continued to lead the globe in floriculture commerce in 2018, accounting for 43.7% of all exports. Demand and manufacturing have also steadily increased in Asian nations including India, China, Thailand, Japan, Sri Lanka, etc. India ranks 14th in the world for exporting floriculture products and is the second-largest flower-growing nation after China. However, India's percentage share in global floriculture exports is only 0.4% in 2018 which might be due to lacunae in maintaining international quality standards, lack of integrated cold chain management and unorganised market and distribution channels (Anumala & Kumar, 2021).

Table 3. Top selling cut flowers in wholesale markets in 2020 (Plantion, Netherlands) (cut flowers by turnover) (AIPH, 2021).

	Turnover (In million EUR)	**Quantity (In million pieces)**	**Average price (EUR/piece)**
Roses	9.6	41.5	0.23
Tulips	4.7	35.2	0.13
Gerbera	2.3	17.8	0.13
Carnations	1.3	11.0	0.12
Eustoma	1.8	4.3	0.42
Paeonia	1.8	4.2	0.42
Chrysanthemums	1.2	3.6	0.34
Alstromeria	1.0	4.9	0.21
Eucalyptus	1.1	3.2	0.35
Lilies	1.1	2.4	0.47
Hippeastrum	0.8	0.9	0.93

* Source: Plantion, 2021

Table 4. Top selling cut fowers, sales fgures (clock and direct sales) in wholesale markets in 2019 (Royal FloraHolland, Netherlands) (AIPH, 2021).

	Quantity (in 2019) (In million pieces)	**Quantity (in 2015) (In million pieces)**
Roses	3 261	3 558
Tulips	1 564	1 802
Chrysanthemums (Ind Grp TR)	1 135	1 150
Gerbera	827	894
Freesia	210	247
Liliies	276	297
Carnations	146	161
Chrysanthemums (Ind Grp GE)	138	157
Alstroemeria	157	146
Eustoma russellianum	235	144
Hypericum	109	112

* Source: Royal FloraHolland, 2020

In terms of sales value, cut flowers dominate the floriculture industry worldwide. Roses, chrysanthemums, carnations, and lilies are the top four floriculture crops in terms of global trade value. Some floriculture production has shifted to countries in the Southern Hemisphere as a result of the requirement to produce enormous amounts of high-quality flowers all year long with lower labor and other production costs. The productivity in these nations is expected to rise even higher, and the global production of floriculture products will continue to increase, particularly in new producing countries in Asia, Africa, and Latin America (Xia et al., 2006).

There are important stakeholders in both established and developing nations, and their relationships are deeply intertwined. Flowers and plants are distributed largely through the Netherlands, which today facilitates or transits 45% of global trade (Union Fleurs, 2022a).

The umbrella organization for floriculture trade enterprises (cut flowers, foliage, and pot plants), Union Fleurs (International Flower Trade Association), was established in 1959 and is situated in Brussels. It has members in 20 different nations. Of the more than 3.000 enterprises represented by Union Fleurs, 1.500 are traders and wholesalers of floriculture in the European Union. More than 80% of the value of the global trade in cut flowers and potted plants is accounted for by members (Union Fleurs, 2022a).

The only years where there have been small declines in floriculture corporation exports and imports globally are 2015 and 2016. In 2017 (the most recent year available), the global exports and imports of floriculture totaled USD 8.7 billion and USD 8.2 billion, respectively (Adebayo et al., 2020).

3. MAJOR CUT FLOWER MARKETS

Major cut flower producing countries are China, US, Netherlands, Germany, Italy, Colombia, Japan, Spain, France, India and Ecuador.

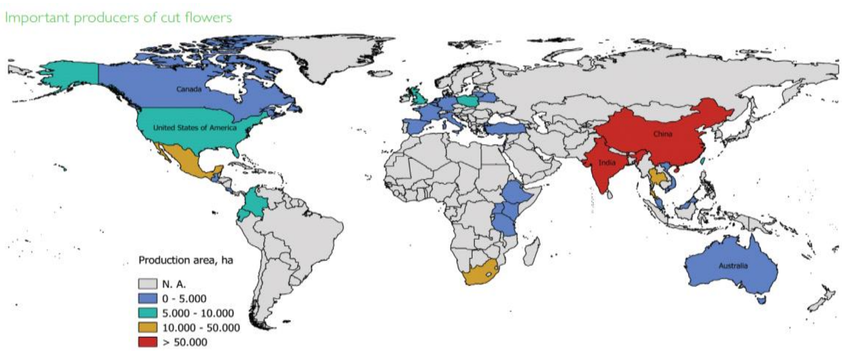

Figure. 1. Important producers of cut flowers (AIPH, 2021)

3.1. China

Farmgate value surged during the past ten years, rising from over €8 billion in 2006 to almost €20 billion in 2016. Only 10% of imported fresh cut flowers are from Thailand, Ecuador, and the Netherlands, respectively. Given the enormous domestic commerce figures, the €8 million in 2016 export value of cut flowers from China may be almost totally ignored. Online flower businesses like RoseOnly and PandoraFlora are benefiting from the explosive development in online sales. On Taobao, the biggest online marketplace, there are also a lot of flower vendors. 45 cities with a population of over a million people are included in the four largest urban areas. The combined population of these four Metro Clusters is currently 471 million, or 34% of all of China.

By 2030, this population is expected to increase to 573 million, or 39% of all of China. In 2017, their combined consumer spending on flowers and plants was €16 billion (40 percent of all China), and it is anticipated that this amount will increase to €57.5 billion by 2030. (AIHP, 2019a).

MOTIVATION	THE MOST POPULAR TYPE OF FLOWER FOR THE PARTICULAR MOTIVATION
Go to hospital	Flower & Fruits basket, Lily
Showing love	Rose
Apologise	Rose
Anniversary	Rose
Buddha	Sleeping Lotus
Store Opening Day	Lily, Rose, African Daisy
Mother's Day	Carnation, Rose
Teacher's Day	Carnation
Tree-Planting Day	Carnation, Sunflower
National Day	African Daisy, Sunflower
Valentine Day	Rose

Figure. 2. Cut flower varieties by consumption purpose, China, 2016 (AIHP, 2019a)

Flower species such as Rose, Lily and Carnation have the highest online sales volume. The flower species such as Baby's breath, Forget-me-not, Sleeping Lotus, Sunflower, Blue Enchantress, Eustoma grandiflorum, Eucalyptus, Desmodium Gyrans and Fortune Chloranthus herb are also very popular (AIHP, 2019a).

3.2. India

Cut flowers for export have replaced traditional blooms in the Indian floriculture sector. The liberalized economy has encouraged Indian businesspeople to set up export-focused floriculture facilities in climate-controlled environments. India's floriculture industry is being developed and is being promoted for export by the Agricultural and Processed Food Products Export Development Authority (APEDA). Cut flowers, pot plants, cut foliage, seeds, bulbs, tubers, rooted cuttings, and dried flowers or leaves make up the majority of the items produced by floriculture. Rose, Carnation, Chrysanthemum, Gargera, Gladiolus, Gypsophila, Liatris, Nerine, Orchids, Archilea, Anthurium, Tulip, and Lilies are significant floricultural crops in the global cut flower trade. In greenhouses, flowers such as gerberas and carnations are produced. Chrysanthemum, Roses, Gaillardia, Lily Marygold, Aster, Tuberose, Crossandra, China aster, etc. are some of the crops grown in open fields. Major floriculture hubs have formed in Maharashtra, Karnataka, Andhra Pradesh, Madhya Pradesh, Haryana, Tamil Nadu, Rajasthan, and West Bengal. In 2020–2021, 322 thousand hectares of the land were being farmed for floriculture. In 2020–21, the production of flowers is expected to reach 828.09 thousand tonnes of cut flowers and 2151.96 thousand tonnes of loose flowers. According to the National Horticulture Board's National Horticulture Database, India's floriculture production area in 2020–21 was 322 thousand hectares, producing 828 thousand tonnes of cut flowers and 2152 thousand tonnes of loose flowers. Several states now practice commercial floriculture, with Kerala (16.5%), Tamil Nadu (13.3%), Karnataka (11.4%), Madhya Pradesh (11.1%), and Uttar Pradesh (7%) outperforming other producing states like Andhra Pradesh, West Bengal, Mizoram, Gujarat, Orissa, Jharkhand, Haryana, Assam, and Chhattisgarh in terms of area. The Indian floriculture sector produces flowers like marigolds, roses, tuberoses, glads, anthuriums, and roses. In addition to modern poly and greenhouses, open farm settings are used for cultivation (APEDA, 2022).

India's extensive biodiversity is well-known. The need for a coordinated effort in focused research and development program to develop new hybrid varieties suitable for the country's various agroclimatic conditions, involving several Institutions of Excellence in developing climate-specific hybrids in addition to other ornamental native species for both cut flowers and pot plants, as well as their cultivation methods and packages (Hegde, 2020).

One of the top producers of young plants for cut flowers, potted plants, and vegetable seeds worldwide in India is Florance Flora. Among a select group of health-conscious consumers, edible flowers including citrus blossoms, hibiscus, lavender, roses, and nasturtium are becoming more popular. These blossoms are abundant in vitamin C and antioxidants, which lower cholesterol levels, promote the health of the skin and hair, and improve heart function. The worldwide floriculture market offers new potential for producers to grow their operations due to the rising popularity of flowers as a healthy food ingredient. Among product type, the cut flowers segment is considered a key contributor to growth of the global floriculture market, due to high consumption of fresh roses in decorations such as bouquets at events, vase arrangements, and as gifts on special occasions (FMI, 2022).

3.3. USA

According to the U.S. Bureau of Economic Analysis' Personal Consumption Expenditures, sales of floral products at all retail locations totaled $31.3 billion in 2015. (2016). According to consumer trends, 34% of people purchase fresh flowers. 24 percent of the overall wholesale value comes from Florida. California (21%) and Michigan (11%) are the states that follow Florida in terms of popularity. Domestically grown cut flowers had a wholesale value of $295 million in 2020, which was a 10% decrease from 2019. With a wholesale value of $203 million, California is the top state in the US for producing cut flowers, making up 69 percent of the value of the cut flowers produced by the other top states (USDA, 2016).

Tulips and Lilium make up the majority of cut flowers, with the remaining flowers being imported at a cost of €1.7 billion annually. Weddings and funerals are two significant life occasions in which flowers are culturally ingrained. Even if the market is vast and developed, certain parts are growing, which provides opportunity for novel ideas. Although the number of individuals in working age is not increasing as quickly as either of the other two consumer categories in North America, their worth is increasing. Because of the logistical and labor issues that growers must deal with, there may be some shortages and an increase in retail costs (AIHP, 2019b).

The United States has become more dependent on imports as domestic production of cut flowers has declined over the past few decades. Since Colombia typically holds a 60% market share in the USA, it was in an excellent position to seize that market (AIHP, 2019c).

Figure. 3. Field grown flowers (AIHP, 2019c)

One of the biggest producers of nursery plants and floriculture crops worldwide is the United States. According to the Floriculture Crops 2018 Summary report (USDA NASS, 2019), all farmers in the US with sales of $10,000 or more were expected to generate a total wholesale value of $4.63 billion in 2018. (Khachatryan & Wei, 2021).

3.4. EU

The major actors are spread out and highly intertwined. Flowers from several sources can be combined into one bouquet for decoration (EU and non-EU). $15 billion was traded in cut flowers globally in 2015. More than 50% of global trade passes through or is exported again via the Netherlands (Union Fleurs, 2022b).

The EU has one of the highest densities of flower production per hectare in the world thanks to rising levels of flower production and decorative plant farming. The EU has a net trade surplus for live plants and floriculture products but is a net importer of cut flowers and cut foliage (EC, 2020).

3.5. Colombia and Ecuador

Naturally, Colombia and Ecuador, two global leaders in the production of cut flowers, are located in Latin America. The advantages of location, altitude, labor force, and other economic factors have fueled the increase of the quantity and price of cut flowers produced in these nations. The majority of Colombia and Ecuador's exports historically go to North America, with smaller amounts going to Europe and Russia, but overall, these two nations serve the global market, and their flowers have traveled to every corner of the globe either directly or via re-export through hubs like the Netherlands, for instance (AIHP, 2019b). 90% of the US market is supplied collectively (Columbia 65%, Ecodor 25%). 90% of imports arrive at the Miami airport (Union Fleurs, 2022b).

3.6. Kenya

One of the major foreign exchange earners for this nation is the export of ornaments, which brought in little about €663 million in 2016. With a market share of over 38%, Kenya is the top exporter of rose cut flowers to the European Union. Although direct export destinations are becoming more significant, over 65% of exported flowers are sold through Dutch auctions. Around Lake Naivasha, Mount Kenya, Nairobi, Thika, Kiambu, Athi River, and Nakuru are the main production locations. The speed of export documentation processing and phytosanitary handling are identified as growth inhibitors. Enhancing Kenya's connections to the global airfreight network and expanding the quantity and capacity of direct flights to the key markets would provide Kenya more opportunity to stand out from the competition. Using a lot of water to produce flowers puts a strain on farms' operational models. Furthermore, conflicts arise between member farms of the Kenya Flower Council (KFC) and nearby communities that have historical claims to land and water resources. These conflicts arise as a result of drought and population growth in the main growing regions. 95% of Kenya's cut flower exports come from the Lake Naivasha Basin (AIHP, 2019b). Kenya provides one-third of the cut flowers sold in the EU (roses). exports to more than 50 different countries worldwide. Over 2 million people's livelihoods are impacted, and 10,000 people directly and 50,000 indirectly depend on the floriculture business (Union Fleurs, 2022b).

CONCLUSİONS

Main cut plant species are Roses, Tulips, Chrysanthemums, Gerbera, Freesia, Liliies, Carnations, Alstroemeria, Eustoma russellianum, Hypericum, Gypsophila, Paeonia, Limonium, Zantedeschia, Anthurium, Hippeastrum, Hydrangea, Gladioli, Iris, Snapdragons, Delphinium, Orchids, Lisianthus, Cymbidium, Leatherleaf Ferns and Sunfower.

Key Countries are China, US, Netherlands, Germany, Italy, Colombia, Japan, Spain, France, India, Ecuador, Poland, Kenya, UK, Ethiopia, Portugal, Costa Rica, South Africa, Brazil, Thailand, Mexico, Iran, Australia, Turkey, Vietnam, Israel, Malaysia and Korea Republic

Major cut flower wholesalers are FloraHolland (NL), Landgard (DE), Veiling RheinMaas (DE), OTA Floriculture Auction (JP), FAJ Flower Auction Japan (JP), Veiling Holambra, São Paulo (BR), Plantion (NL), Taipei Flower Auctions (TW), New Covent Garden Flower Market (UK), SS Flora Koop Istanbul (TR), UFG United Flower Growers, Burnab (CA), Euroveiling Brussels (BE), Multifora Johannesburg (ZA), SICA Marché aux Fleurs, Hyeres (FR), United Flower Auction (NZ), Ontario Flower Growers (CA), Flora Max (NZ) and UC Flor San Remo (IT).

Key Companies are Forest Produce Ltd., Selecta Cut Flowers S.A.U., Native Floral Group, Tropical Foliage Plants Inc., Oserian Group, Esmeralda Farms, Marginpar BV, DOS GRrINGOS LLC, Flamingo Horticulture Ltd., Danziger Group, Florensis Flower Seeds UK Ltd., Verbeek Export B.V., Florance Flora, Kariki, Multiflora, Karen Roses, Harvest Flower, Queens Group, Ball Horticultural, Afriflora, Karuturi Global Ltd., Oserian, Selecta

One, Washington Bulb, Arcangeli Giovani & Figlio, Carzan Flowers, Rosebud, Benary, Danziger, Sakata, Oserian, Selecta One.

Direct sales of floral products, sales through specialty stores, sales through franchises, sales through florists and kiosks, sales through supermarkets and hypermarkets, sales through independent small stores, and sales through online retailers are the main sales channels for the floral industry.

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CHAPTER 11

BIOTECHNOLOGICAL USE, BIODEGRADATION CAPACITY AND PATHOGENICITY OF ASPERGILLUS SPECIES IN DIFFERENT ORGANISMS

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INTRODUCTION

The first definition made by P. A. Micheli was made with the description of the characteristic spore-bearing structure of the genus Aspergillus (Perrone & Gallo, 2017). Nearly 400 species of members of the genus Aspergillus have been discovered so far, with four subgenus and 19 divisions. These microorganisms are living things that can colonize living and non-living organisms from soil to animals, from plants to humans. Due to such a wide variety of hosts, the effects caused by members of this genus include medicine and veterinary (pathogenicity of *A. fumigatus* and *A. terreus* species to animals and humans), agriculture and food (*A. flavus*, *A. parasiticus*), and industry (*A. niger*, *A. aculeatus*, *A. oryzae*) (Samson et al., 2014). As one of the most frequently isolated fungal species, Aspergillus is found in more than half of the total isolations with 51-86% (Alastruey-Izquierdo et al., 2013; Escribano et al., 2013; Fischer et al., 2014; Mortensen et al., 2011). Although *A. fumigatus* is the most frequently isolated and most common species in the genus Aspergillus, this species is followed by *A. flavus* or *A. terreus*, *A. niger* and *A. tubingensis* in decreasing order (Alastruey-Izquierdo et al., 2013; Baddley et al., 2009; Burgel et al., 2012; Fischer et al., 2014; Jensen et al., 2016; Mortensen et al., 2011; van der Linden et al., 2011). However, it is also known that there are some Aspergillus species whose places in the classification have not been fully resolved yet, and these are estimated to constitute approximately 40% of the total antifungal Aspergillus (Alastruey-Izquierdo et al., 2013; Balajee & Marr, 2006; Posteraro et al., 2011; Vinh et al., 2009).

Biotechnological Use of Aspergillus

Aspergillus species, as mentioned earlier, are found in many places in nature and can be toxic to humans, animals and plants in various ways. On the other hand, some species of these fungi are also used as biotechnological factories in biofilm studies (Knuf & Nielsen, 2012; Seidler et al., 2008). The products obtained by biofilm studies are used to combat various stress conditions (Cray et al., 2015). In addition to their biofilm properties, Aspergillus strains are used in the production of various enzymes (eg: amylase), in the production of various fermented foods (eg: soy sauce), in the synthesis of some organic acids such as citric acid and the production of some drugs (Perrone & Gallo, 2017).

In addition to other uses, fungi are frequently mentioned in the treatment of environmental pests (eg pesticides). Unlike physicochemical treatments, biological treatments have proven to be environmentally cheaper and more efficient and have become increasingly widespread as an eco-friendly system. It has been observed that pesticides can be degraded by microorganisms in soil and at high pH (Racke et al., 1996). Similarly, *A. terreus*, one of the Aspergillus species, was also shown to be involved in this process (Singh et al., 2003). Thus, it is predicted that soils can be cleaned cheaply and efficiently by the biological method, which is an environmentally friendly method, without physicochemical processes.

The human population is increasing day by day. This increasing population requires a continuous increase in resources or the widespread use of sustainable resources. Areas of sustainable sourcing include the reuse of waste-generated products as raw materials, which include cellulose-containing paper waste and lignocellulose-containing agricultural waste. Although they do not attract attention as waste, these materials are very valuable as they are a source of organic fertilizer and animal feed (Paramjeet et al., 2018; Ruqayyah et al., 2013). Recycling lignocellulose sources using fungi is highly advantageous compared to other recycling methods (Abdel-Hamid et al., 2013; Abou-Shanab et al., 2012; Fernandez-Fueyo et al., 2016). Toxic by-products can be produced in the recycling process and thus they are far from being an environmentally friendly method. Filamentous fungi play a critical role in the degradation and recycling of plant biomass due to their saprophytic properties and their high enzymatic activity potential.

One of technological application of Aspergillus species is their use in cellulose digestion. It is known that some fungi belonging to the genus Aspergillus are also producers of cellulolytic enzymes (Payne et al., 2015; Su et al., 2016). Lignin-degrading fungi do this by providing extracellular enzymes that enable them to do so by converting lignin into smaller intermediates/intermediate products (Beckham et al., 2016). *A. niger* (Rawat et al., 2015) is known to be one of the organisms active in cellulase production in this fungal group. The cellulase production potential of *A. tubingensis* NKBP-55, another member of the Aspergillus family, was also found by isolating the fungi in samples taken from cow manure and investigating their properties (Prajapati et al., 2018). *A. flavus* EGYPTA5 was also considered an efficient non-cellulolytic lignin-degrading fungus (Hasanin et al., 2019).

One of the materials that are very difficult to degrade is polyethylene (PE) and other materials originating from them. Microplastic particles originating from and consisting of these themselves are gradually ceased to be an environmental problem and enter the list of materials that threaten animal and human health. The study by Zhang et al. in 2020, on the other hand, shows that *A. flavus* obtained from moth excrement can decompose these microparticles, which cannot be degraded naturally (Zhang et al., 2020). An example of the biotechnological uses of *A. flavus*, one of the Aspergillus species, is alternative uses in textile wastewater. When suitable conditions (such as pH, and temperature) are provided, *A. flavus* has been shown to decolorize effectively in textile wastewater (Selim et al., 2021). In addition, the phytotoxicity of these wastewaters was also examined by inoculating these textile waters and the pod germination was carried out, and the effectiveness of the study was confirmed. Therefore, in addition to the degradation of cellulose and polyethylene by *A. flavus* fungi, it is also encountered as an environmentally friendly method in wastewater treatment.

Aspergillus Pathogenicity

Another feature of some members of the genus Aspergillus is that they are pathogenic to other organisms such as plants, animals, and humans. Aspergillus, which is widely found in nature, is very important for the carbon and nitrogen cycle, the existence of infections due to Aspergillus has also been studied as pathogen/mold formation in the living body since the beginning of the 19th century (Mayer, 1815). Although they differ according to the immune status of the host organism, they can cause a wide variety of findings both in terms of clinical symptoms and the types of diseases they cause. When a list is made in terms of pathogenic species of Aspergillus, *A. fumigatus*, *A. flavus*, and *A. niger* are included in order according to the frequency of causing pathogenicity (Marr et al., 2001). As an example, *A. fumigatus* is one of the most important airborne pathogens (Blatzer & Latge, 2017). This feature of *A. fumigatus*, which is listed as the most pathogenic, has been shown to result from the evolutionary ability that allows it to easily adhere to the host organism/environment (Tronchin et al., 1993). A. fumigatus, a saprophytic fungus that produces volatile conidia (spores) in the air, is the organism that causes various fungal diseases, including allergies and aspergillosis, in cases where it is inhaled by humans and the conditions are suitable (excess or lack of immune system responses)(Klich, 2007). It has been shown that the resistance that occurs in patients against azole therapy used against this human pathogenic microorganism is caused not only by azole derivatives used in the clinic but also by fungicidal agents used against the agricultural pest Aspergillus (Berger et al., 2017; Hagiwara et al., 2016).

While some of the species commonly found in plants show saprophytic properties, they can be found on newly developing plants and damage them before harvest. *A. niger*, which can be given as the only example of this species, seriously damages some fruits and vegetables and has serious effects on their harvest and consumption (Pitt & Hocking, 2009). In addition, spoilage caused by aflatoxin contamination can develop in plants consumed as food in a way that affects the qualitative, sensory, or nutritional value. They cause deterioration of food, bad smell, change in pigmentation, change in taste, causing damage to the agricultural economy by decreasing purchasing rates, and also causing damage to animal and human health if they are consumed unnoticed.

Mycotoxins and Aspergillus

Many fungal species, including the genus Aspergillus, produce secondary metabolites for various purposes such as maintaining their lives, communicating, and competing for food. Some of these secondary metabolites may be pathogenic for other living things. *A. niger* strains with known pathogenic properties in plants even if they rarely produce mycotoxins (Cabanes & Bragulat, 2018; Pitt & Hocking, 2009). However, members of the genus Aspergillus, which is known to have close to 400 species, can be found almost everywhere in tropical regions. Although there is population-specific variation even within aflatoxin-producing species (Drott et al., 2021), three species of Aspergillus fall into the aflatoxin-producing group when their ability to produce mycotoxins is taken into account. According to the review of Tainwaki et al., these are *A. flavus* and its close relatives; *A. ochraceus* and close relatives; and *A. carbonarius* and the closely related *A. niger* (very rarely) (Taniwaki et al., 2018). It has been shown that, unlike other aflatoxin-producing relatives of *A. flavus*, which have a commensal life in peanut and maize plants, they can cause infection of these plants under certain stress conditions (such as drought stress) and thus cause them to be contaminated with aflatoxin. Other aflatoxin-producing species have been reported to produce postharvest mycotoxins (Pitt et al., 2012).

Due to the effects of conditions such as increasing global warming, carbon dioxide and sunlight, the reproduction environments of fungi that cause toxicity are increasing, and therefore their rate of toxin production increases (Medina et al., 2017). According to a study by Gilbert et al. in 2018, it was shown that *A. flavus* infection in maize increases depending on the amount of water, temperature and carbon dioxide and affects many cellular pathways (Gilbert et al., 2018). Considering this increasing and continuing situation of global warming, it is feared that the formation of suitable environments for toxin-producing organisms will be inevitable and even more resistant and productive organisms will emerge with the recombination that will occur during their reproduction.

As a plant pest, fungi can cause a wide variety of diseases. These include septoria leaf spot, powdery mildew and rust (Price et al., 2015). To eradicate these organisms, a series of antimycotic applications are made and they are tried to be destroyed by external intervention. In addition to antifungal (eg: azole) application, it is tried to prevent contamination from *A. flavus* with strategies such as adding various microorganisms to the environment and making the primary spread of these microorganisms or adding the substances secreted/derivated by these organisms to the environment. According to a study conducted in 2018, it was shown that *A. flavus* AF1B production decreased by adding *Saccharomyces cerevisiae* itself or cell wall isolates to pistachios (Abdolshahi et al., 2019). Similar to this feature of *S. cerevisiae*, which is a single-celled fungus, studies have shown that some lactic acid bacteria stop fungal growth (Sangmanee & Hongpattarakere, 2014).

Differences in aflatoxin production were observed in terms of factors such as the phenotype of sclerotia production (Abbas et al., 2005), geographical origin (Razzaghi-Abyaneh et al., 2006), vegetative compatibility (Novas & Cabral, 2002) and genetic diversity (Acur et al., 2020) among aflatoxin producing species within the genus Aspergillus. These feature differences also enable biological control of toxic species by using non-toxic strains (Okoth et al., 2018). Beyond these, plants have developed several defense mechanisms by their immune systems against their invaders. Against infectious fungi such as *A. flavus*, the resistance (R) genes in the host plant come into play, triggering a series of reactions that will initiate the signaling that will protect the host. The peculiarity of these genes is their specificity to recognize the invasive species specifically, and thus their ability to perform species-specific defence. The response resulting from these initiated signal reactions is called the hypertensive response (HR), and by targeting the slowdown of fungal growth, it is resisted by creating localized lesions that trap the fungi (Chisholm et al., 2006; Greenberg, 1997; Piffanelli et al., 1999). Different from this biotrophic pathway, a different defense system has been developed for necrotrophic fungi. In this defense system, it is seen that the plant activates the Jasmonate and ethylene signaling pathways rather than the species-specific defense, and adds the whole system to the defense (Oliver & Ipcho, 2004). Looking at previous work on mapping populations in maize, several quantitative trait loci (QTLs) associated with resistance to *A. flavus* have been identified (Brooks et al., 2005; Warburton et al., 2009). With this and similar studies, it is becoming increasingly important to identify genes that play a role in the development of resistance or susceptibility to invaders and to develop resistant lines against the related disease or pathogen by using these genes. In this way, it will reduce the agricultural effects of these pests, provide pathogen-free food to humans and animals, and thus prevent diseases.

Use of Bioactive Compounds to Cope with Aspergillus

Especially today, the increasing rate of contamination in agricultural products and the increase in global warming push scientists to search for more effective methods in the fight against fungi. The use of chemical control agents, which can now be described as old methods, is an increasing concern due to the damage they cause to the environment, and most importantly, the microorganisms they fight begin to develop tolerance to these agents (Tripathi & Dubey, 2004). Therefore, there is increasing interest in creating more environmentally friendly fungal control strategies.

Among the plants that have started to be used effectively in the fight against fungi, there are various herbs, spices and aromatic plants known to have strong antimicrobial effects. The limitation of the use of these plants is that the content compositions change due to factors such as the environment in which they are grown, various environmental stresses and pH changes. In addition to this variable feature of the chemical content, they also have a strong odor, high volatility, low water solubility and unstable structures, even if their antimicrobial properties are high, their use may be limited (Kumar et al., 2019).

One of the new alternatives that seem likely to replace synthetic fungicides used as chemical control agents is the antifungal use of essential oils, which are generally responsible for the strong odor of aromatic plants (Bakkali et al., 2008; Lang & Buchbauer, 2012). Although the antimicrobial effects of essential oils are known beforehand, recent studies also reveal their capacity to be effective antifungals (Burt, 2004)(Burt-Tisserand). The antifungal and anti-aflatoxigenic responses of essential oils obtained from the thyme plant against *A. flavus* by inhibiting mycelium growth and aflatoxin production have been shown in various studies (Bluma & Etcheverry, 2008; Kohiyama et al., 2015; Omidbeygi et al., 2007). According to a relatively recent study by Oliveiraa et al., thyme oil's ability to disrupt the integrity of the plasma membrane and induce apoptosis lies behind the antifungal effect strategy of thyme oil (Oliveira et al., 2020). It was also shown in the same study that the reason behind the inhibition of aflatoxin production was the downregulation of the laeA gene and the modulation of the hydrolase gene (which provides colony formation).

Although cumin is a plant that is widely used as a spice and therefore harvested by various countries, it is also widely consumed in various digestive system problems due to its content (Allahghadri et al., 2010). In addition to the use of this variety, it has been shown that the essential oil obtained from cumin also has a restrictive effect on the growth of Aspergillus species; therefore, cumin has an antifungal effect (Kedia et al., 2014; Khosravi et al., 2011). It has been understood that the feature that gives the functionality of cuminaldehyde, the active ingredient of cumin, comes from the effect of *A. flavus* on membrane integrity, triggering necrosis and downregulation of genes related to the secondary metabolism of *A. flavus* (Xu et al., 2021). It has been shown biochemically that the essential oil obtained from the dried leaves of rosemary, another plant widely used as a spice, also has antiaflatoxigenic properties and inhibits mycotoxin production to a large extent (Bomfim et al., 2020).

Cinnamon, one of the other spice plants, has been found to suppress aflatoxin production through secondary compounds it contains, similar to the previous spices (Wang et al., 2019) It has been discussed that the reason for this decrease in aflatoxin synthesis is that cinnamaldehyde, one of the compounds found in cinnamon, causes regulation in transcription, downregulating the genes responsible for aflatoxin production, and also upregulating oxidative stress-related genes. Studies made from essential oils obtained from cloves, another frequently used spice after cinnamon, also show that essential oil obtained from cloves has antifungal, antibacterial and anti-inflammatory properties, as in other types of spices (Das et al., 2019; Lee et al., 2020; Luo et al., 2019). It has also been reported that the encapsulated forms of this oil have an inhibitory effect on aflatoxin production and therefore they can be used for biocontrol purposes (Kujur et al., 2021).

It has been revealed by the transcriptomic study that another plant-derived agent is the essential oil of *Perilla frutescens* (Hu et al., 2021). In this study, it was observed that some genes in *A. flavus* were suppressed as a result of the intervention with *Perilla frutescens* essential oil, and the cell wall and morphology of *A. flavus* and various metabolic pathways were changed.

Moreover, access to raw materials and low recognition of the raw material (Bucar et al., 2013), the gradual decrease in biological diversity (Prakash et al., 2015), the amount of volatile compound isolated from the raw material and its rapid degradation (Turek & Stintzing, 2013), while the antifungal activities of essential oils can be seen strongly in vitro, various agents that carry them in vivo (nanocapsulation) are being developed to overcome this limitation. For this reason, encapsulation of plant-based products has become increasingly important and various encapsulation methods have been tried by working with some Aspergillus species. For example, liposomes and nano-gels for *A. flavus* (Beyki et al., 2014; Zhaveh et al., 2015); nanoprecipitation for *A. parasiticus*; Solid lipid nanoparticles have been studied for *A. ochraceus*, *A. niger*, *A. flavus* and various studies have shown that encapsulated essential oils have better antifungal and anti-aflatoxicogenic effects.

In addition to plant-based bioactive components, various bioactive substances (extracellular vesicles, peptides) obtained from some bacterial species have also been found to have antifungal properties. According to a study conducted in 2020, low-weight peptides produced by *Lactobacillus plantarum* TE10 were shown to inhibit the growth of *A. flavus* on maize seeds (Muhialdin et al., 2020). In another study using lactic acid bacteria, it was observed that four different lactic acid bacteria produced antimicrobial compounds (various dipeptides, phenolic compounds) in the presence of *A. flavus* (Cortes-Zavaleta et al., 2014; Ruggirello et al., 2019) and were able to degrade mycotoxins (Elsanhoty et al., 2013; Sangsila et al., 2016).

In addition to bacteria, non-toxic forms of fungi can be used to control biological fungi to reduce or eliminate the effects of toxic forms (Du et al., 2017; Xu et al., 2015). As an example, when the activity of *Eurotium cristatum* strain against *A. flavus* was tested, it was observed that it could inhibit the growth of toxic *A. flavus* and inhibit its aflatoxigenic properties (Zhao et al., 2020). It has been reported that secondary metabolites produced by *Eurotium cristatum* strain are especially responsible for these activities. In the aforementioned study, both the inhibition of fungal growth was determined by monitoring the damage to hyphae using biochemical data, and the genes responsible for aflatoxin production (aflD, aflQ and aflS) were shown to be down-regulated using genetic data.

Biocontrol can be done by using *A. oryzae*, which is effective in fungal control and is also a genus of *A. flavus* in the fight against *A. flavus* (Alshannaq et al., 2018). The emergence of this discovery was that *A. oryzae*, a non-toxicological member of the genus Aspergillus, which is used to ferment some foods, does not contain *A. flavus* contamination in foods (such as soy, miso, soy sauce) fermented. In the light of the data obtained by Alshannaq et al. in 2018, it was observed that when *A. oryzae* was grafted on peanuts with even 1%, it could replace the toxic (Alshannaq et al., 2018) *A. flavus* species. Moreover, the fact that both cellular and non-cellular culture filtrates of this fungi inhibited aflatoxin production. Thus, it was concluded that the secondary compounds secreted by this species are the main mycotoxin production inhibitory agents.

CONCLUSION

In this review study, the aim is to focus on the biotechnological importance, pathogenicity and environmentally friendly methods of dealing with the toxic species of the genus Aspergillus by taking the studies of the last 5 years to the fore. Due to the widespread usage of *A. flavus* from effects on food to effects on human health, studies on this species have been mainly taken into account.

With the widespread use of fungi for useful activities such as enzyme studies, it has been observed that Aspergillus strains can also be used for this purpose. For this purpose, in recent years, quite a lot of information has begun to be accumulated. On the other hand, factors such as changing climatic conditions and environmental pollution have irreversible consequences on living species. For this reason, the tendency towards environmentally harmless or relatively less harmful agricultural practices and drug applications with less adverse effects on human and animal health is increasing. As mentioned in this review, it is likely to be an environmentally friendly application in many areas, from synthetic substances such as polyethylene glycol degradation of Aspergillus species to reducing mycotoxin production by synthesizing natural secondary compounds.

Various studies have shown that it is possible to keep toxic Aspergillus species under control even without chemical and physical applications, by biological methods. It has been found that the use of essential oils is effective in reducing Aspergillus toxicity and aflatoxin production. However, due to the various limitations of essential oils mentioned in the article, it has been observed that researchers continue their research by developing nanoencapsulation strategies. In addition to herbal extracts, some bacterial and fungal cells and isolates have been shown to have similar activity.

In summary, the high diversity of Aspergillus strains expands both the beneficial use of this genus and its toxicity. In addition, changing conditions pave the way for the reproduction and spread of toxic strains, causing economic damage and increased health problems caused by Aspergillus. However, improvements will be made in favour of non-toxic species with the development of especially environmentally friendly methods of coping with them.

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CHAPTER 12

DIFFERENTIALLY EXPRESSED GENE ANALYSIS IN CANCER RESEARCH BY USING BIOINFORMATIC TOOLS

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INTRODUCTION

Genome-wide screening of transcriptional changes between normal cells, cancer cells, and cells in the metastatic processes provides information about the molecular basis of various cancer type. With the help of bioinformatic analyses, personal approaches can be adopted in the diagnosis, treatment, and survival processes of various cancer types including frequently observed and rare cancers. Performing bioinformatic analyses before clinical and laboratory applications provides great advantages in terms of time and money. In this book chapter, genomic-level screening of differentially expressed genes performed by using bioinformatic tools in different cancer types that support pan-cancer genomic studies will be summarized.

1.1 The Concept of Differentially Expressed Gene Analysis

All somatic cells of an organism have the same genome, but starting from the embryonic development processes, each cell and cell group differentiates to display different functions with changes in the genome. Differentiation for different purposes is dependent on the different gene expression levels specialized for specific functions in the genome (Shiraki et al., 2014). In addition to differentiation, some genes may be expressed in a different way than they would normally be, with the effect of different stimuli in disease states. There are biochemical processes in a cell that determine which genes are actively transcribed and whether they are translated into mRNA and proteins. In addition, under certain conditions, the expression of these genes (upregulated or downregulated genes) changes under certain conditions during pathogenesis such as cancers (Fattahi et al., 2019; Moreira et al., 2008; Lin et al., 2014). In case of cancers, Differential Gene Expression (DEG) analysis of both RNA and DNA microarray data/RNA-Seq data/cDNA microarray data and determined differentially expressed genes (DEGs) are used to clarify the different gene status between healthy and disease states (primary cancers, metastasis status, etc.). To understand the difference, quantitative gene expression-based changes between control and experimental groups are compared to each other by performing statistical analysis with the help of normalized data (Fang et al., 2012; Dudoit et al., 2002; Storey et al., 2003; Bullard et al., 2010).

1.2. Approach to Cancer in terms of Differentially Expressed Gene Levels and Pan-Cancer Studies

Determination of gene expression signatures/profiles in the formation of cancers provides important information about biological phenotyping and biological pathway-related processes. In the literature, there are many valuable studies in which individual genes or small gene clusters deviating from normal changes (such as mutation, expression profiles) are shown in different cancers.

There are more than 200 different types of cancer identified to date. In cancers, various types of genetic alterations such as somatic mutations, altered gene expression levels, epigenetic aberrations are observed (Tomczak et al., 2015).

If we focus on breast cancer, many studies have shown that breast cancer cells differ from normal cells at the gene level. Besides non-genetic factors such as physical activity, obesity, menstrual background and alcohol usage, genetic based predispositions play important role in breast cancers. Mutations can be classified into three classes such as high penetrance mutations (TP53, BRCA1-2, P53, PTEN genes, etc.), moderate penetrance variants (ATM, CHEK2, BRIP1 genes, etc.), low penetrance variants (FGFR2, TOX3, MAP3K1, COX11, NOTCH2/FCGR1B genes etc) (Antoniou et al., 2008; Birch et al., 2001; Nelen et al., 1996; Renwick et al., 2006; Meijers-Heijboer et al., 2002; Seal et al., 2006; Easton et al., 2007; Ahmed et al., 2009; Thomas et al., 2009). Furthermore, oncogene activation (such as Human epithelial receptor 2, (HER-2), c-myc, p-53) and tumor-suppressor gene inhibition (p27, Skp2, breast cancer susceptibility gene 1 and 2 (BRCA1,2), PTEN, Retinoblastoma (Rb), etc.) can be observed in breast cancer development which have been identified various valuable research studies (Osborne et al., 2004).

As can be noticed in the breast cancer studies summarized above, in addition to the determination of mutations of individual genes or the determination of gene expression levels, pan-cancer studies conducted with big data also support these studies in a wide scope. The use of patient gene expression data obtained from clinical applications gives us the opportunity to work with more heterogeneous patient groups, and this enables more effective diagnosis and treatment process planning that can be both personalized and generalized in cancer patients. For this reason, The Cancer Genome Atlas (TCGA) and the International Cancer Genome Consortium (ICGC) projects were conducted in 2005 and 2008, respectively (https://www.genome.gov/Funded-Programs-Projects/Cancer-Genome-Atlas) (Chin et al., 2011; Tomczak et al., 2015). The Cancer Genome Atlas (TCGA) project and TCGA Pan-cancer Clinical Data Resource (TCGA-CDR) provides genetic alterations for almost 33 cancers with 11,000 tumour gene data which can be used to determine the survival rates of patients (Liu J et al., 2018).

1.3. Differentially Expressed Gene Analysis in Cancers

Although the genetic (mutation accumulation, genome instability, gene expression levels (up-regulated or down-regulated genes), etc.) and biological mechanisms (tumour triggered inflammation, invasion and metastasis, deregulation of energy systems, escaping cell death, increasing the proliferation capacity, etc.) that occur in cancer tumorigenesis are standard, each patient's cancer status and cancer-related diseases are different from each other (Senga et al., 2021; Hanahan et al., 2022). Therefore, although there are common processes, cancer is on its way to becoming a personal disease. Starting from this point, DEGs can be determined by using big data showing patient gene changes and gene densities. Thus, using multiple data and bioinformatics tools and making gene-based screening provides us with more comprehensive data in a short time. Gene expression data at the genome scale provides a profile of differently expressed genes that can distinguish between different biological states. This abnormally differentiated gene profile can be used successfully to assess prognosis, chemotherapy status, and drug sensitivity in a tumour sample (Stevenson et al., 2012).

There are studies aiming to investigate the DEGs relevant to prognosis of various cancers. To achieve this, integrated bioinformatics analyses are used with the help of different computer-based and online bioinformatic tools.

1.3.1. Bioinformatic Based Tools used in Patient Gene Data Collection for Differentially Expressed Gene Analyses

To determine DEGs, gene intensity data and microarray data can be downloaded from publicly free databases such as Gene Expression Omnibus (GEO) Database developed by National Center for Biotechnology Information (NCBI) and The Cancer Genome Atlas (TCGA) developed by the National Human Genome Research Institute (NHGRI) and the National Cancer Institute (NCI). In this book chapter, we will summarize the NCBI GEO database.

The GEO project was initiated in response to the need to store multiple gene expression data. For example, with the help of storing and grouping the data obtained from gene expression experiments (such as microarray or RNA-Seq experiments) specific to cancers, it is possible to compare cancer to normal with datasets including gene expression data of related genes (Edgar et al., 2002; Barrett et al., 2012).

Publicly available gene data (in here GEO data) can be analysed by using statistical platforms such as R software program. R-based GEO data analysis can be achieved by using web application such as GEO2R (https://www.ncbi.nlm.nih.gov/geo/geo2r/) (Barrett et al., 2012). Furthermore, it can also be used via downloading free R software which is also widely used in statistical computing area (https://www.r-project.org/). There are packages such as BioConductor, repository for bioinformatics software, which can be used to compare at least two groups (such as samples and controls) to determine which genes are differentially expressed. BioConductor is a project and there are developed BioConductor packages (such as Limma for microarray and RNA-Seq data) which can be used by using R statistical programming language (https://www.bioconductor.org/) (Ritchie et al., 2015). Furthermore, there is a BioConductor package named as RankProd which can be used to detect DEGs in meta-analysis (Hong et al., 2006)

1.3.2. Determination of the Biological Relevance and Gene Enrichment Analysis

Upon determination DEGs (up-regulated or down-regulated genes in cancer patients), these DEGs can be enriched in different biological processes. To make biological sense of the data obtained from the analyses made by means of bioinformatic tools in which hundreds or even thousands of gene expression data are used, it is necessary to use bioinformatic tools and to make sense of these complex processes. For this purpose, bioinformatic tools providing gene-annotation enrichment analysis service have been developed. Thanks to these tools, it is possible for researchers to identify the biological processes, functions, and pathways most suitable for their studies. Some of these bioinformatic-based tools and free databases are available and these databases that can be used without requiring an ethical permission procedure. For instance, The Kyoto Encyclopedia of Genes and Genomes (KEGG) is a type of analysis that uses information from genome and gene data. Eventually, signalling pathways that can interact at the functional and molecular level are identified (Kanehisa, 2002). KEGG pathway analysis can be achieved by using web-tools specified for this purpose. For instance, Gene Ontology enRIchment anaLysis and visuaLizAtion tool (Gorilla) (http://cbl-gorilla.cs.technion.ac.il/) and ShinyGO v0.741 (http://bioinformatics.sdstate.edu/go74/) tools are frequently used to predict enriched GO terms (DEGs) and relate these comprehensive gene lists to particular biological process, function or component. The Database for Annotation, Visualization and Integrated Discovery (DAVID) is another web-based tool which is used by researchers to understand the biological relation of high number of differentially expressed genes (https://david.ncifcrf.gov/). Furthermore, KOBAS web-based analysis database is a tool where KEGG analysis can be performed by using DEGs lists (http://bioinfo.org/kobas). It has two modules named as "annotation module" and the "enrichment module" and these modules are used to annotate the GO terms and relate the biological pathways respectively (http://bioinfo.org/kobas). Onto-tools is known as a toolkit to be used as “Onto-Express”, “Onto-Translate”, ” “Onto-Design”, “Onto-Compare” and (Draghici et al., 2003). The Signalling Gateway Molecule Pages (SGMP) is a database where the interaction between the analysed proteins and signal transmission pathways can be determined (Dinasarapu et al., 2011). The STRING database is publicly available to predict and identify the interacting genes and determine the protein/protein interaction (PPI). For this reason, the database contains the data of 14094 organisms and 67.6 million proteins and can exert more than 20 thousand interactions (https://string-db.org/). Furthermore, there are other web-based tools such as MAPPFinder, GoMiner, EASE, GeneMerge and FuncAssociate, GENEONTOLOGY, PANTHER Classification System which can be efficiently used in bioinformatic analyses (Huang et al., 2009).

1.3.3. Drug Sensitivity and Drug Resistance Prediction via DEG Analysis in Cancers

DEG analysis can be conducted to determine the drug sensitivity and resistance in human diseases. For that reason, Connectivity Map (CMAP) database, which was funded by NIH LINCS (Library of Integrated Cellular Signatures) project, has been developed and it supplies broad range data also for cancer studies (https://www.broadinstitute.org/connectivity-map-cmap) (Lamb et al., 2006; Nevins et al., 2007). In this project, mostly cell line-based and patient data-based studies have been conducted to determine gene expression patterns upon the interaction of small molecules and certain drugs such as estrogen receptor agonists and antagonists, HDAC Inhibitors, Phenothiazine, Gedunin, Sirolimus, etc. The most frequently used cell lines are breast cancer cell line (MCF7), leukaemia cell line (HL60), melanoma cell line (SKMEL5) and prostate cancer cell line (PC3) in this project (Lamb et al., 2006). In addition, Lee Y.S. et al. has been identified acquired gefitinib resistance (AGR) related hub-DEGs through network analysis (meta-analysis) in lung cancer and epidermoid carcinoma by using PC9 and A431 cell lines, respectively (Lee et al., 2015).

1.3.4. Determination of Hub-Genes and Survival Prediction via DEG Analysis in Cancers

Hub-genes can be predicted via DEG analysis in different cancers such as papillary thyroid cancers, breast cancers, lung cancers, cervical cancers, hepatocellular carcinoma, kidney cancer (Sun et al., 2021; Xiao et al., 2018; Xue et al., 2020). Furthermore, DEG analysis can also be conducted to predict survival related hub-genes in certain cancers. For instance, Zhu et al. (2019) has been shown that autophagy pathway related 16 DEGs are involved in survival process in multiple myeloma (MM) patients. They propose that autophagy related gene prognostic model can be considered as a basis of anticancer therapies in MM patients (Zhu et al., 2019). Similarly, autophagy based DEG signature with 3 autophagy-related genes (SQSTM1, BIRC5, and FOXO1) and its effect on survival rates has been identified in hepatocellular carcinoma patient groups (Lin et al., 2018). In another cancer type named as gastrointestinal pan-adenocarcinomas, alternative splicing pattern of multiple genes has been predicted and their impact on prognosis and survival of these cancer patients have been identified (Lin et al., 2018). Besides, podocan, which is a regulatory protein in extracellular matrix (ECM), encoding gene PODN has been considered as a biomarker for osteosarcoma patients in both diagnosis and prognosis processes. Furthermore, including PODN, the most significant 5 genes (PODN, OLFML2B, ACTA2, COL6A3, FAP, and COL6A1) have been determined as significantly and differentially expressed genes and they are related with the survival of these osteosarcoma patients (Yao F., et al. 2021). In gastric cancers, Wang et al. (2015) has tried to predict differentially expressed miRNA patterns as a biomarker via meta-analysis and they have determined the effects of these miRNAs on both survival and treatment responses (Wang et al., 2015).

1.3.5. Metastasis Status Prediction via DEG Analysis in Cancers

DEGs analysis can also be done to predict the metastasis status of cancers. For instance, Qi et al. (2019) has predicted that there are more than 1000 differently expressed and methylated genes (677 genes upregulated-hypomethylated, 361 downregulated-hypermethylated) which are related with certain pathways linked to tumorigenesis and metastasis in breast cancer patients (Qi et al., 2019). Similar study has been conducted by using aggressive breast cancer cell line gene expression data and Chen et al. (2015) has predict the metastasis related DEGs (such as PTX3, SNAI2, IL-8/6, etc.) and related biological processes (such as tyrosine metabolism, calcium signalling pathway, etc.) (Chen et al., 2015). On the other hand, colorectal cancer originated liver metastasis related DEGs have been predicted by Liu et al. (2021) by using gene expression data of cancer patients and they have determined that cell adhesion molecules are the molecules which should be focused on, and peroxisome proliferator activated receptor (PPAR) signalling pathway is the key biological process in their study (Liu et al., 2021).

CONCLUCSION

Gene expression profiles and DEG analyses that allow hub-gene identification will become much more relevant once prospective and clinical laboratory-based studies are performed and data are validated. This will assist clinicians to routinely use microarrays to better diagnose and predict cancers and enhance the prognosis of cancer patients.

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