ADVANCED STRATEGIES FOR AGRICULTURE

II

EDITOR

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PREFACE

As we stand at the intersection of technological innovation, environmental sustainability, and the ever-growing demand for food production, the agricultural landscape is undergoing a profound transformation. This book, "Advanced Strategies for Agriculture," emerges as a guiding beacon in navigating the complexities and challenges faced by modern agriculture.

In an era where traditional farming practices are meeting the frontiers of cutting-edge technologies, the need for a comprehensive understanding of advanced strategies has never been more critical. This book delves into the intricacies of next-generation farming methods, precision agriculture, and sustainable practices, offering a holistic perspective for both seasoned farmers and newcomers to the field.

The journey through these pages takes us from the fertile grounds of tried-and-true agricultural principles to the uncharted territories of data-driven decision-making and artificial intelligence in farming. Each chapter is crafted to provide not only theoretical insights but practical applications, ensuring that the reader is equipped with the tools needed to thrive in a rapidly evolving agricultural environment.

We extend our gratitude to the experts and pioneers who have contributed their knowledge and experiences to this volume. Their dedication to pushing the boundaries of what is possible in agriculture is evident in the wealth of information presented within these chapters.

As editors, we hope this book serves as a catalyst for innovation, a source of inspiration, and a reference guide for all those passionate about advancing agriculture. Whether you are a farmer seeking to optimize your yields, a researcher pushing the frontiers of agricultural science, or a policymaker shaping the future of food security, this book is designed to be a valuable resource in your journey.

We invite you to explore the pages that follow, delve into the advanced strategies presented, and join us in the exciting endeavor of shaping the future of agriculture.

Happy reading and may your fields be abundant!

CHAPTER 1

SUSTAINABLE AGRICULTURAL PRODUCTION: PERSPECTIVE ON WHEAT AND DRY BEAN – WORLD AND TURKEY

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INTRODUCTION

Increase in the population and higher life standards are further exacerbating the scarcity of water resources that are used in agriculture. Therefore, the increasing of demand in food poses a major problem for security for the food. For this reason, it is extremely important to address the problem among providing and claim for water resources. Additionally, fast social growth eliminated the cycling of carbon in continental ecosystems, causing an increase in the amount of CO_2 gas in the atmosphere and in temperature values on a global scale. Desertification, drought and land destruction due to climate change and the failure of the ecosystem cycle due to them cause social and economic problems and pose increasing risks to agriculture over time (Wu et al., 2021). Considering global water resources, 71% of existing water resources are used in the agricultural sector (The World Bank, 2019).

Functional foods are defined as foods or food components that are effective in reducing the risk of disease and achieving a healthier life by fulfilling one or more beneficial functions in the human body, in addition to meeting the basic nutritional needs of the human body (Sevilmiş, 2013; Cömert and Gün, 2020).

A wide variety of functional foods have been developed in recent years. These are: probiotic, prebiotic and synbiotic foods, foods with reduced fat, reduced salt and reduced sugar, and foods enriched by adding some substances (Gasmalla et al., 2017).

Those taken from outside with food are generally called phytochemical antioxidants. They are biologically active compounds and have strong antioxidant activity. For this reason, they have protective and antimutagenic properties against viral diseases, cancer and allergies, and are effective in preventing many chronic diseases. Fruits, vegetables and whole grains have very high antioxidant content (Bagdi et al., 2016).

High fat consumption is associated with type-2 diabetes, increased risks of stroke and heart disease. Many countries have established guidelines to reduce fat consumption (Paximada et al., 2021). For developing regions, demand for food is outgrowing at an annual rate of 1% and changes as 170 kg for Central Asia region to 27 kg for Eastern and Southern Africa regions. In developing parts of the world (Central Asia and China), wheat accounts for approximately 53% ratio for entire lands and half of the production (Shiferaw et al., 2013).

Today, population growth, global crises, natural disasters and rising food prices have further increased the importance of the agricultural sector in meeting food needs. Factors such as climate change, decrease in agricultural product stocks, price increases in energy and other inputs, population growth, and the development of the use of agricultural products in alternative fields such as biofuel led to excessive increases and fluctuations in food prices in the second half of the 2000s. It is expected that the demand for agricultural products will increase and food prices will remain at high levels, especially in developing countries, due to population and welfare increases (KB, 2014).

Animal and plant-derived proteins are the two main sources of protein in human nutrition. Excessive amounts of animal-derived proteins lead to diseases such as obesity, cardiovascular diseases and high blood pressure (Lin et al., 2017). As for plant proteins, it has been observed that it has antimicrobial, anti-cancer, lowering blood glucose and blood pressure, and positive effects on bone and cardiovascular health (Carbonaro et al., 2015).

It is necessary to understand how plants perceive the environment and organize their signaling networks. Perception of stress and the plant's response to this stress occur through signal transduction pathways (Garg and Kumari, 2016).

The higher the level of genetic variation in the initial population in a plant breeding program, the wider the genetic base and the higher the success rate (Nachimuthu et al., 2015).

When selecting genotypes with good physical characteristics in breeding programs, environmental conditions such as rainfall, soil structure and plant nutrients contained in the soil should also be taken into consideration, as well as the genetic structure (Dziki and Laskowski, 2005).

The world population is increasing rapidly and it is estimated that the population will be approximately 50% more than today in 2050 and will exceed 10 billion by 2100, and it is claimed that the food deficit will increase day by day (Nellemann et al., 2009; Anonymous, 2011).

Wheat (*Triticum* spp.), is the oldest and most important, most produced and consumed of the plants in the cereal group, and is a strategic plant as well as being an economically important plant in the agricultural sector and throughout the world. In ancient civilizations, the use of wheat for bread purposes was less than barley, but the fact that wheat is easier to separate from its seed coat and is more suitable for bread making has increased the preference of the wheat plant (Sheaffer and Moncada, 2012).

Despite changes in consumption and nutrition habits, wheat will continue to be a plant that maintains its strategic importance due to its ability to be a basic nutrient (Kaya, 2009). Wheat is among the most consumed grains worldwide, with a production of approximately 750 million tons every year, a cultivation area covering 220 million hectares and providing 15% of daily calories (Balfourier et al., 2019).

In terms of food security, the food and energy needs of one third of the world's population are met from wheat. It is reported that the origin of pasta and bread wheat species are wild species growing in the area called "Fertile Crescent", which includes the Southeastern Anatolia Region of Turkey (Baloch et al., 2016; Aktaş et al., 2018).

Dry bean (*Phaseolus vulgaris* L), the most consumed among the legumes group, are one of the leading alternative protein sources. Dry beans are consumed as a basic protein source, especially in underdeveloped countries. Therefore, the cultivation of dry beans is concentrated in Asia and South America (FAO, 2021).

Legumes are the seeds or fruits of plants belonging to the family *Leguminaseae* or *Fabaceae*. The word legume comes from the Latin “Legumen” and means seeds harvested from shelled broad beans. Pulse (pulse) is derived from the Latin word "puls" and means porridge or jelly (Sarıoğlu and Velioğlu, 2018).

To date, current efforts in research in the field of hybrid seeds have been limited, with breeding programs not exploiting the full potential of genetic resources and productivity gains through their use consistently stagnating (Sharma et al., 2015).

It has been stated that the reason why ancient wheat species are decreasing day by day is due to the higher productivity and more profitability of modern bread and pasta wheat varieties with the breeding program, although ancient wheats are generally used in low-scale farming practices (Shewry and Hey, 2015).

The fact that legumes are the most suitable products for both climatic conditions and environmental management plans of countries, and that legumes can be used for various markets, including fish feed, animal feed and export, apart from human consumption, will increase the legume cultivation areas. However, due to container shortages and increasing freight prices, and congestion at ports, countries have difficulties in supplying dry beans and transporting products from the field to processing centers (BUGEM, 2022).

Determining management strategies for land and water resources used for agricultural purposes is an extremely complex phenomenon, as well as having many objectives. It is essential that the authorities making high-level planning on the subject take into account all components of agriculture (ecology, economy, social) in a planned and continuous manner in order to ensure better agricultural development (Lence et al., 2017).

In this study, wheat and beans are discussed in terms of sustainable agriculture and food safety, and a summary of the current literature on the subject is presented.

PERSPECTIVE ON AGRICULTURAL SUSTAINABILITY

“Sustainable Agriculture; it can also be defined as "directing agricultural activities in a way that protects productivity and the environment in the long term, ensures economic development, and increases the quality of rural life." (Çeker, 2016; Süzer, 2021).

Although organic agriculture may seem like an environmentally focused production system at first glance, it has now become an important economic activity both in the world and in Turkey. Today, the volume of the world organic product market has reached 120.6 billion Euros (Willer et al., 2022).

The materials obtained by collecting many varieties of wheat from around the world, including those that have not yet been determined, have been protected in gene centers by morphological descriptions and evaluated in breeding studies of those showing high-level characteristics in terms of agricultural activities (Köksel et al., 2016).

It is possible to reduce the significant greenhouse gas effects caused by basic grain types (such as wheat, corn, rice) by reducing nitrogen-containing chemicals and implementing more sustainable management in large-scale enterprises. Although some strategies help eliminate abundant organic carbon and nitrogen in soils and thus greenhouse gases (Sun et al., 2019).

While human crises may not always result in environmental crises, environmental crises almost always result in human crises (WWF, 2013). Soil is the storage-source where the most important nutritional elements in nature are transformed, it is the most important ecosystem between air (atmosphere), water (hydrosphere), rocks (lithosphere) and living things (biosphere), forming the living space for humans and living things (WWF, 2021).

“Natural disasters such as floods, fires and droughts experienced on a global scale in recent years threaten the ecosystem, especially human life, there is an increase in the frequency, impact and duration of these disasters. Climate change is no longer an environmental problem, but rather a sustainability problem. "It is now essential to take the necessary precautions against climate change to ensure the living standards of future generations” (TASAV, 2022).

Soil is the main source of carbon and provides almost 95% ratio for the world food production (Anonymous, 2019). Soil is the second store of carbon after the oceans, so ensuring sustainable use of soil means helping to reduce the presence of carbon dioxide in the atmosphere, thereby reducing the greenhouse effect (Zucaro and Morosini, 2018).

For wheat and dry beans, which are the most widely used species in the world for nutritional purposes, it is of great importance that agriculture in large areas around the world adopts sustainability principles for both soil and other ecological components

PERSPECTIVE ON WHEAT BY VIEW OF SUSTAINABILITY CONCEPT

The grains we use in our diet, especially wheat, which is the raw material of our basic food, bread, must be rich in nutritional content. Wheat is a strong source of antioxidants due to the phytochemicals it contains (phenolics, tocopherols, carotenoids, etc.), and with these antioxidant properties it contains, it prevents and delays the progression of many chronic diseases (Menteş Yılmaz, 2011).

Belonging to the *Poaceae* family, wheat is an allohexaploid species that occurred as a result of successive natural hybridizations in the Fertile Crescent throughout the Neolithic period, approximately 8000 - 10000 years before (Sansaloni et al., 2020). It is known that wheat is the most important basic energy source in developing countries, therefore its nutritional quality needs to be improved (Barut et al., 2019). As an average, almost 44% for everyday energy comes from bread alone, besides 53% is provided from by bread and altered grain-based products (Şanlıer, 2013).

Wheat is the largest contributing plant, with approximately 30% of world grain production besides almost 50% ratio for trade of cereals over the world (Akter and Rafiqul Islam, 2017). Wheat is the main food source of approximately 50 countries (Kılıç, 2009). The first wheat cultivated, *Triticum monococcum* L. subsp. *monococcum*, it was cultivated during the Pre-Pottery Neolithic Age in southeastern Turkey (Zaharieva and Monneveux, 2014).

It has been emphasized that of the 100% increase in wheat yield and quality, 60% is a reflection of new breeding varieties with high yield potential and 40% is a reflection of developments in cultural practices (Bulut, 2012). In arid and semi-arid regions, the yield of wheat grown depending on rainfall decreases significantly due to insufficient water resources (Wakchaure et al., 2016).

It has been reported that the grain yield, harvest index, thousand grain weight and number of ears per square meter of the varieties increased in irrigated conditions compared to barren conditions, while plant water use efficiency and protein ratio decreased (Zeleke and Nendel, 2016). It has been explained that the most important factor for the quality criteria of wheat in irrigated and arid areas is the variety (Souza et al., 2004). To ensure the high degree of genetic diversity required for selection processes in breeding programs, a core and representative germplasm collection is required (Matus and Hayes, 2002; Mathew et al., 2019).

In the 1950s, mechanized agriculture increased, techniques such as tillage depth, time and shape in fallow systems were developed, and chemical fertilizers began to be used in wheat agriculture and soil fertility increased. Since previously improved varieties were developed for poor conditions, they have partially or completely adapted to these conditions, and have grown in agronomic conditions and growing environments suitable for high yields because they have weak stems and are tall. Therefore, in addition to adaptation to bad conditions, importance has also been given to the use of fertilizers, advanced agricultural techniques and the development of varieties that are suitable for irrigated conditions and have a high response to fertilizer (Özberk et al., 2016).

During the period called the green revolution in the world between 1940 and 1970, the spread of short-tall, high-yield and high-quality wheat varieties caused a decrease in wheat production areas in the world, but an increase in yield and production amount (Morgounov et al., 2019). The Norin 10/Brevor variety, which was brought to Turkey from Mexico in 1967 under the influence of the period called the green revolution, which included the transfer of dwarfism genes to wheat, was hybridized with local wheat in Turkey and produced hybrids such as Sonora-64, Pitik-62, Penjamo-62, 7C and Super x and so great progress has been realized (Özberk et al., 2016).

Consumed by billions of people, wheat is a staple food in many diets. Based on the increase in worldwide mortality attributable to diet-related chronic diseases, there is increasing interest in identifying wheat species with greater health potential, more specifically enhanced antioxidant and anti-inflammatory properties. In addition to being an important source of starch and energy, wheat provides significant amounts of components that are essential or beneficial to health, especially protein, vitamins (especially B vitamins), dietary fiber, and phytochemicals (Dinu et al., 2018; Zheng et al., 2019).

Wheat provides 20% of the daily protein intake for developing countries and is the primary source of protein for the world's population

(Braun et al., 2010). The quality of protein in a food depends on the amount of protein it contains, as well as the number and amount of essential amino acids it contains, as well as its digestibility (Bhat et al., 2021). Many processes such as agricultural practices, genetic structure, milling and cooking processes contribute to the end-use quality of wheat (Güçbilmez et al., 2019). It has been stated that the variance of environmental effects for protein ratio is greater than the variance of genetic factors (Peterson et al., 1992). Today, the final limits of genetic variability that can be used to solve production and quality problems in grains, which are of vital importance for human nutrition, have been approached. Genetic gains in yield are currently around 1% per year (Mackay et al., 2011).

Wheat has a strategic importance among grain products because it is easily and cheaply available compared to other foods in terms of protein and carbohydrates (Duru et al., 2019). Wheat and products derived from wheat constitute a large portion of the energy needs in our diet, as well as containing significant amounts of protein, carbohydrates and phytochemicals. These valuable phytochemicals they contain are of great importance because they reduce the risk of contracting diseases and have a protective effect (Ryan et al., 2011). Wheat, one of the most important cultivated plants in the world, spread to different geographical areas on earth, is the basic food item in people's nutrition and provides approximately 2% of the calories taken from food (Toklu and Yağbasanlar, 2005).

Although there has not been any important change for farming areas of wheat in the last 40 years, and there was always an increase in the amount of production due to the use of higher yield varieties and certified seeds, as well as increased irrigation, fertilization, pesticide opportunities and appropriate cultivation techniques, production in the targeted quantity and quality has not been achieved (Sonkurt, 2018; Güvercin, 2019).

One of the most effective growing techniques in increasing the yield and quality characteristics of wheat is fertilization (Kızılgeçi et al., 2016). Only 10-20% of the fertilizer phosphorus applied to agricultural soils can be used by plants (Gupta et al., 2020). It is seen that organic fertilizer applications increase the organic carbon and soil fertility of the soil and thus provide a higher yield tendency compared to a balanced chemical fertilizer (Zhang et al., 2014; Scaglia et al., 2016). Foliar fertilization is cheaper and more efficient than soil fertilization and requires less fertilizer (Singh et al., 2015). While there is an average of 95 kg of fertilizer used per hectare in Turkey, the average use of fertilizer per hectare in the world is 116 kg, and in the European Union and other developed countries, this rate is over 200 kilograms (ZMO, 2019).

It has been reported that there is a sulfur deficiency problem in many of the world's agricultural lands today (Camberato and Casteel, 2017). When sulfur is deficient in the growing environment, the productivity and quality of plants decrease tremendously, causing a decline in protein synthesis and a significant decrease in the content of sulfur-containing amino acids such as cysteine, cysteine and methionine (Marschner, 2011; Chahal et al., 2020).

Conscious fertilization is essential for sustainability. Sulfur deficiency in wheat reduces the concentrations of protein and amino acids in the wheat grain and worsens the bread-making properties of wheat flour (Raffan et al., 2020). Wheat seeds were primed in 25, 50, 75, and 100 μM sodium selenate solution for half and one hour at 25°C and the grain amino acid content was examined (Nawaz et al., 2012). Due to pre-treatment with 75 μM solution for one hour, a significant increase in the amount of sugar and total free amino acids was observed.

Wheat ranks first in breeding studies, especially because it contains essential amino acids (Ranum et al., 1990). Methionine, threonine and tryptophan, especially lysine, are the limiting amino acids in grains (El, 2016). The effect of sulfur on protein and amino acids in winter wheat was examined (Jarvan et al., 2008). According to the research results, when sulfur consumption increased compared to the control, an increase was observed in the amount of protein and amino acids. This increase rate was calculated as 24.5%, 35.3%, 14.4% and 7.7% for cysteine, methionine, threonine and lysine, respectively.

Insufficient nutrition of plants with nutrients reduces their ability to survive and fight against diseases and pests, weeds, and their resistance to stress factors such as heat, drought and cold. Nutrition directly affects crop yield and quality in economically important cultivated plants (Yadav et al., 2020).

Considering that drought will increase in the future, resistance studies against drought and other stresses should be increased, especially by making use of genetic resources, and studies on developing high-yield and high-quality varieties should be increased (Daryanto et al., 2016; Sarto et al., 2017; Zhang et al., 2018). Genetic diversity in crops such as wheat has decreased over the years due to intensive selection breeding using limited genetic populations (Cowling, 2013).

A variety of quality testing procedures continue to be used to characterize wheat for different end uses, ranging from alveograph, farinograph and cooking tests (Huen et al., 2018). Today, agronomic biofortification (fertilizer application) and plant breeding are the two most important methods to increase the microelement content of grains (Pfeiffer and McClafferty, 2007; Cakmak et al., 2010). Wheat grain contains approximately 65-75% starch, 8-15% protein, 11-13% water, 1-5% fat, 1.5-3% sugar and 1-2% ash. In addition to carbohydrates, fat and protein, there are other vitamins, especially B vitamins that play an important role in human and animal nutrition (Özdemir et al., 2012).

Since the phenolics in wheat can scavenge free radicals and prevent radical damage that may occur in DNA, RNA, proteins and cellular organelles in human health; has become the center of attention. However, phenolic compounds found in wheat and other grains are predominantly found in bound form. This causes them to not be easily extracted in organic solvents (Chen et al., 2015). It has been stated that durum wheat varieties with high yellow pigment value are determined by their superior properties and that the color value is very important in the commercial, nutritional and technological quality evaluation of durum wheat (Banach et al., 2021). Vacuum and short-time kneading systems used by the pasta industry in recent years require high protein quantity and quality. This should be taken into consideration in breeding studies and hybridization programs should be created in this direction (Pehlivan and İkincikarakaya, 2017). Since the gluten structures of soft biscuit and top wheat are weak, low sedimentation values are obtained in the analysis (Karaduman et al., 2020).

Compared to wheat flour, wheat flour has 3 times more protein, 7 times more fat, 15 times more sugar and 6 times more mineral content. In addition, it is thought to be a food very similar to eggs, which are rich in plant-based proteins. Although the protein it contains has a high availability rate, it is closer to animal-derived proteins in terms of biological value (Demir et al., 2019). Protein content, which is very important for textural properties, can reflect the nutritional level in wheat flour and have a significant impact on dough rheological properties (Brankovic et al., 2018).

The length of the wheat grain is approximately 8 mm and its weight is 30-40 mg (Köksel, 2019). Pericarp, or fruit shell, is the outer part of the wheat grain. It covers the entire grain surface, is 50 microns thick and constitutes nearly 5% of the grain. It contains approximately 20% cellulose, 6% protein, 2% ash and 0.5% fat, with the remainder being non-starch polysaccharides (Delcour and Hoseney, 2010).

Although it is known that the iron content of an average wheat grain is in the range of 25-35 mg kg^{-1}, this value is insufficient to meet people's daily iron needs (Rengel et al., 1999, Çakmak et al., 2010). Wheat grain consists of 3 parts: endosperm, embryo and seed coat. Bran and seed coat, which are rich in fiber, are found in approximately 14.5% of the grain and contain niacin, vitamins B1 and B2 and pantothenic acid. Endosperm is the starchy part of the wheat grain and is used in flour production. Its proportion in the grain is approximately 81-84%. The embryo, which constitutes 2.5-3.6% of the wheat grain, contains more protein and fat than the other parts (Bilişli, 2012). The activity of dietary fibers is known as prebiotic activity (Mudgil, 2017).

It has been stated that genetic and environmental factors affect the mineral content of wheat (Ciudad-Mulero et al., 2021). It has been reported that the effect of genetic factors for gluten is broader than environmental factors (Peterson et al., 1992). Separation of gluten protein into its various components is important for comprehensive studies in molecular breeding and better selection of desired properties for higher gluten quality (Kiszonas and Morris, 2018).

The reason why wheat has such a wide cultivation area and production in the world is that this plant has a wide adaptability, is easy to cultivate, has relatively low input needs and can meet a very significant part of human' daily calories (Morgounov et al., 2019). Demand for wheat in global scale has rised owing to grow in total population, change in food menus related with habits and also changes by view of social - economic conditions, particularly for Asian and African countries (Mondal et al., 2016). In the research conducted by the United Nations Food and Agriculture Organization, it was determined that 690 million people were not adequately nourished (FAO, 2020). According to USDA 2020/21 production season projections, wheat production constitutes 28% cereal production for the world, that is equal to 2.7 billion tons amount, meantime exports of wheat formed by 42% ratio, that is equal to 464 million tons quantity (USDA, 2022). Due to the insufficient domestic production capacity in many countries, the place of agricultural products in foreign trade is also very important (Aysu, 2018).

According to FAO's estimate, the world will need a production level of approximately 840 million tons by 2050, down from the current 770 million tons of wheat production level. This demand excludes the need for animal feed and the negative effects of climate change on wheat production. To meet this demand, it is estimated that developing countries will increase their wheat production by 30% and the world will need an additional 70 million tons of wheat by 2050 to meet future demands (Sharma et al., 2015).

Since its adaptability is very high, it can spread over wide areas. In the world, it is cultivated between 20-65^{o} north and 22-45^{o} south latitudes (Erekul, 2017). High temperatures during the development period of wheat cause changes in grain size, starch amount and protein ratio, reducing the quality. Deterioration in quality also affects dough properties (Erekul and Yiğit, 2018). 779 million tons of wheat is produced worldwide due to its high growth rate, wide adaptability, being a productive plant and meeting the nutritional, commercial and socio-economic needs of many countries (USDA, 2022).

Protein ratio is directly linked to environmental factors, climate and fertilizer applications, as well as variety, and it has been reported that the protein ratio in wheat varies between 6% and 25% (Anonymous, 1990). In addition to diseases, environmental factors such as climate, soil structure and fertilization literally prevent the wheat from maturing and filling and cause the weight of hectoliter to decrease (Mutlu and Taş, 2020).

Wheat, barley, corn, rice, etc., which are considered as basic products in ensuring food security in the world. The grains produced are products of strategic importance for many countries and regions in the world. Supply of grains, it is highly dependent on production and stock situations, and production fluctuates from year to year. In the period 2004-2014; World grain production increased by 22%, grain supply by 24%, grain use by 22% and grain trade by 34%. It is seen that world grain production, which was approximately 2.1 billion tons in 2004, increased to 2.5 billion tons in 2014. Although there was a 22% increase in world grain production in this ten-year period, there were significant fluctuations in grain production from year to year. For example, in total grain production compared to the previous year; There was a decrease of 0.8% in 2009, 0.4% in 2010 and 2.1% in 2012. This decrease continued in 2014, reaching 0.1% ratio (FAO, 2014).

In China, the world's largest wheat producer, per capita wheat consumption is below 100 kg. Likewise, average wheat consumption per capita in European countries is below 100 kg (FAO, 2021). While the EU, Russia, USA and Ukraine, which are the important producing countries in world wheat exports, are at the top, Argentina and Turkey are also among the important wheat exporting countries. According to official data, Turkey has the 9th place in global scale by 6.1 million tons amount for wheat exports during the 2019/20 poduction season (USDA, 2022).

Wheat is an annual herbaceous plant and among the nearly 12 thousand plant taxa distributed in Turkey, it is among the plants that have attracted the most attention for both scientific and socio-economic reasons. Turkey is home to more than 20 wild wheat species and more than 400 improved wheat varieties (UHK, 2011). Wheat, which forms the basis of field agriculture in Turkey and can be grown in almost every region; Due to its features such as diversity of usage areas, fully mechanized agriculture, availability of support purchases and ease of cultivation, it ranks first in terms of cultivation and production with 14.5 million tons of production in approximately 5.5 million hectares of land (TUIK, 2022). While Turkey ranks third in the world's pasta production, it is the world's second largest pasta exporter after Italy and exports to 160 countries. Unlike other producing countries, all pasta factories in Turkey produce the semolina required for pasta production themselves (Göymen, 2022).

In Turkey, the use of the primitive form instead of the wild form of wheat took place for the first time in the Southeastern Anatolia Region, namely Şanlıurfa Gaziantep Diyarbakır. Among the volcanic basalts in the region, einkorn, gernik and other wild wheat varieties, dating back from past to present, each have a different story (WWF, 2016).

Consumption of foods derived from wheat ranks first in Turkey. Although Turkey's wheat yield has increased compared to the 2000s, the average yield is below the world wheat yield. While Turkey's wheat production was 21.5 million tons in 2017, this value decreased to 20.0 million tons in 2018. While the decline continued in the past years, the situation did not change in the 2022 data and fluctuating decreases (19.75 million tons) continued (TUIK, 2022).

In the last 20 years, wheat cultivation area in Turkey was recorded as 7.5-9.8 million hectares and production amount as 17.2-22.5 million tons. Wheat, which is grown in every region of Turkey, is most commonly produced in the Central Anatolia Region. This order is followed by the Southeastern Anatolia Region and the Marmara Region. The smallest share in production belongs to the Aegean and Eastern Anatolia regions (TUIK, 2020). Looking at the amount of fertilizer consumption used in agriculture in Turkey in recent years, it is estimated that 6.0 million tons of fertilizer was consumed in 2019 and approximately 7.1 million tons in 2020 (TAGEM, 2021).

According to the 2018 Wheat Report, Turkey produces 3% ratio for the global production by means of wheat and following the European Union, the Commonwealth of Independent States, China, India, the USA, Canada, Australia and Pakistan (TMO, 2018).

The annual amount of water consumed in Turkey is 44 billion cubic meters and this water is; 7 billion cubic meters of it is used for drinking water, 5 billion cubic meters for industry, and 32 billion cubic meters for agricultural irrigation (DSI, 2019). 18 million tons of wheat produced in Turkey is used domestically, and the rest is exported as wheat and its products. In the country, 79% of wheat is used by the food industry, 13% by the feed industry, and 8% is reserved for seed (Anonim, 2021). Based on official data, wheat production area in Turkey is equal to 3.2% ratio for global cultivation area of wheat for 2019/20 season. The amount in the mentioned area is equal to approximately 44% of the grain production area for Turkey. (TUIK, 2020).

For a plant cultivated in such large areas, a small contribution to sustainability will lead to big and considerable results.

PERSPECTIVE ON DRY BEAN BY VIEW OF SUSTAINABILITY CONCEPT

Today, due to the restrictions imposed by people's religious beliefs, the increasing number of vegetarians/vegans in society, and health concerns in society, the tendency to consume plant-based proteins rather than animal-based proteins is increasing (Aydemir and Yemencioğlu, 2013).

If the current data on food needs and the increase in population around the world are taken into account, it is predicted that by 2050, there will be a need for more than 60% more food than today. The most reasonable tool to meet more food needs and combat the problem of poverty is to increase agricultural production. (Dibaba, 2015).

Plant origininated proteins are used as a component of many foods due to its nutritional properties, antioxidant activity values and functional properties such as film, foam, gel and emulsion formation (Aydemir and Yemencioğlu, 2013). Owing to these properties of proteins, in addition to their nutritional value, provide stability and improvement in textural properties of the products during storage (Tsoukala et al., 2006).

While legume derives from the Latin word 'legumen', which means the harvested seeds of the shelled broad bean, pulses are derived from the word 'puls', which means porridge. Although legumes have an important place in agricultural trade in the world; The World Health Organization recommends the consumption of pulses due to their positive effects on health, such as preventing obesity and preventing non-communicable diseases such as diabetes, cancer and heart diseases. In addition, their low prices, which contribute to their beneficial health effects, increase the consumption of legumes (Sarıoğlu and Velioğlu, 2018).

Societies around the world that face hunger and malnutrition are deficient in protein-based nutrition because they generally consume carbohydrate-based foods such as corn and rice. For this purpose, legume sources can fill the protein nutritional deficiency in these countries, as they are cheap and easily accessible (Boye et al., 2010). It is known that probiotics reduce blood cholesterol levels, increase body immunity, and have antimutagenic and anticarcinogenic effects (Hegab et al., 2021).

Among plant proteins, grains are low in lysine and rich in methionine, an essential amino acid, and legumes are deficient in the sulfur-containing amino acids methionine and cysteine, but are rich in the amino acid lysine. In order to avoid negative effects due to amino acid deficiency, it is recommended to consume legume and grain proteins together to ensure amino acid balance (Damodaran et al., 2008; Rebello et al., 2014).

The Food and Agriculture Organization (FAO) reported dry bean production rates in 2020 as 43% in Asia, 30% in America, 26% in Africa and 1% in Europe. When we look at the dry bean production in 2020 on a country basis, it is seen that India has 20%, Brazil and Myanmar have 11%, China and the USA have 5%, while other countries have 48% ratio (BUGEM, 2022).

Soybean, a source of vegetable protein, is widely used commercially around the world. Soybean, soy protein isolate and concentrate are used in the production of textured soy protein or soy flour. Due to reasons such as the genetically modified organism problem and allergen risk seen in soy, the tendency to produce legumes other than soybeans as commercial protein sources has increased. Therefore today, peas, which are widely produced in the world as an alternative to soybean, protein isolate, concentrate and flour are produced commercially (Day, 2013).

Although the self-sufficiency rate in legumes in Turkey is high (101%), when we look at the product specificity, these rates are seen to be 83.2% in dried beans, 122% in lentils and 94% in chickpeas. In the light of these developments, it is seen that our self-sufficiency rate in legumes tends to decrease, and there is a continuity problem in dry bean production, as it is the product with the highest contraction in the cultivation area, contrary to the developments in the world, and it is determined as the product with the highest disengagement from production among legumes in this process (Berk, 2016).

It has been reported that old varieties should always be continued in production in order to increase product diversity, create genetic variation and prevent old varieties from disappearing (Jankielsohn and Miles, 2017). The genus *Phaseolus* includes 180 different species of beans. Its diversity is very high with genetic variations depending on where it is planted, and it has many names around the world and is the second most important source of protein, especially in Eastern and Southern Africa. While the grains of this bean are generally consumed by drying, other parts such as leaves and roots are used as animal feed (Betancur-Ancona et al., 2011). It is known as the second most cultivated legume species in the world after soybeans (Rahmati et al., 2018). There are almost 76 species of beans. It constitutes approximately half of the total legumes used for consumption worldwide. In the world scale, totally 5 bean types are grown (Celmeli et al., 2018).

It is stated that 50% of the applied nitrogen fertilization is used by plants, 2-20% is lost through evaporation, 15-25% is fixed by clay minerals in the soil and 2-10% mixes with groundwater (Özkan et al., 2018). In addition to being used as food, beans are an important plant species because they increase the organic matter content in the soil, act as an anchor plant, improve the structure of the soil, increase the amount of nitrogen in the soil, and plant residues can be used in the industrial industry (Kızmaz and Gümüş, 2021). Legumes, in addition to protein, contain starch, hemicellulose, cellulose, fat, they also contain minor compounds such as vitamins and minerals in their structures (Ricci et al., 2018).

Beans offer an inexpensive source of protein and food to alleviate food shortages and malnutrition around the world (Ugvuanyi et al., 2022). Legumes contain 20-30% protein, three times as much protein as rice and twice as much as wheat (FAO, 2016).

In a study, the usage areas of legume products in the food industry were examined (Ertaş, 2013). It is emphasized in the research that the increase in legume production that will occur with the implementation of the "Konya Plain Project", one of the important regional projects for the development of Turkey, will accelerate the development of new products based on legumes.

Protein isolates are used in liquid formulations with their high solubility, they are effective in semi-solid and solid products such as soup, emulsified meat products, bakery products and pasta, with their characteristics like capacity of retention for water-oil, emulsion and gelling properties (Tabtabaei et al., 2019).

Phenolic compounds and flavonoids are found in higher amounts in bean varieties with dark colored seed coats compared to light-colored bean varieties and provide increased antioxidant activity. These compounds, as well as anthocyanins often found in the skin, contribute to the color of red and black beans (Chavez-Mendoza et al., 2018).

Bean, they are legumes rich in protein and fibre, and they contain different amounts of vitamins, minerals and phytochemicals (such as sitosterol, campesterol) depending on their variety (Azarpazhooh and Boye, 2013). Moreover, they are also rich in polyunsaturated fatty acids and carbohydrates in complex form (Reyes-Moreno and Paredes-Lopez, 1993). Palmitic and linoleic acid are predominantly found in all beans, at rates of 32.4% and 36.1%, respectively (Shi et al., 2016).

It is reported that approximately more than 20% of the agricultural production areas in the world are under the influence of salinity (Hasanuzzaman et al., 2013, Yazar and Kaya, 2014, Kang et al., 2017). In other sources, it is stated that approximately 45 million hectares (20%) of the world's 230 million hectares of irrigated land and 32 million hectares (2%) of the rainfall-based agricultural area are estimated to be affected by salinity (FAO, 2005, Morales et al., 2012, Aydinşakir et al., 2015).

Edible legumes require soils rich in lime and humus, rich in phosphorus and nitrogen, with a pH between 6 and 8. Legumes are not only rich in nutritional value, but also make important contributions to the soil where they are grown (Avican, 2019).

Local varieties and adaptation studies have always maintained their importance for reasons such as their role in plant breeding and their use as genitors in the development of new varieties (Azeez et al., 2018; Haliloğlu et al., 2022). Especially high temperature, drought and salinity are abiotic stress factors that significantly affect crop production (Shekari et al., 2017).

In the Himalayas, three bean varieties (Luxmi, SVM-1 and Kentucky Wonder) were sown in two-year trials on 1, 8, 15, and 22 June (Sharma et al., 2013). The seed yield obtained from seed sowing on the 1st of June increased by 23, 77 and 85%, respectively, compared to the other three dates.

In terms of consumption in Turkey, the annual per capita consumption of beans is around 3 to 4 kilograms, chickpeas around 6 kg, red lentils around 4 kg, and peas around 1-2 kg (Gülümser, 2016).

The research conducted with bean producers in the Konya region was conducted to determine the non-economic technical problems faced by farmers in bean farming and to develop appropriate solution suggestions. As a result of the research, it was determined that farmers were inadequate in planting frequency, fertilization, irrigation, disease and pest control practices (Önder et al., 2012).

Plant proteins can be produced using very little chemical fertilizer due to their ability to retain free nitrogen in the air. This allows both to increase the fertility of the soil and to reduce the production costs of producers (FAO, 2016).

CONCLUSIONS

In addition to the economic importance of nutrition in meeting social needs, its historical and cultural value is also a fact that cannot be ignored (Sezgin and Bülbül, 2017). Feeding the constantly increasing population in the world and in Turkey is an important problem and naturally increases the demand for food. On the other hand, agricultural lands are constantly lost due to reasons such as misuse and environmental pollution. This makes protecting agricultural lands and ensuring sustainability even more important (Aznar-Sancheza et al., 2019).

For the philosophy of sustainable life, it is undoubtedly one of the natural resources that can be considered indispensable for the continuity of life, especially for human beings is water resources (Eliasson, 2015; Sepehri and Sarrafzadeh, 2018; Houria et al., 2020; Çadraku, 2021). In addition, water scarcity is increasingly coming due to changes in climate and especially direct and indirect activities of human beings (Zou et al., 2020; Lv et al., 2022).

When examining the challenges to the development and adoption of sustainable agroecosystems, it is not possible to separate biological problems from socioeconomic problems (such as inadequate credit, technology, education, political support and access to public services). In fact, the biggest obstacles to the transition from a high capital/energy-using industrial production system to labor-intensive, low-energy-consuming agricultural systems may be social difficulties and political prejudices rather than technical problems (Altieri, 2018).

An ecologically based adaptation approach has often been more accessible and cost-effective for rural communities; It has also brought with it side benefits such as soil improvement, water regulation, carbon accumulation and livelihood diversification. It is based on leveraging biodiversity, ecosystem services and processes to facilitate the adaptation of people and their livelihoods to the impacts of climate change. It promotes economic, social and environmental sustainability and efficiency with an intersectoral perspective; this includes agriculture, forestry, energy, water, social justice, education and sustainable livelihoods, among others (Seddon et al., 2016).

Agriculture in rural areas has an important place in economies as a sector that meets the essential needs of people. Factors such as the dependence of production on natural conditions, the inelasticity of supply and demand, and the length of the production period are the distinctive features of the agricultural sector. Using information technologies in agriculture integrated with classical methods; is important for sustainability. Especially; renewable energy and carbon emissions, vertical farming - soilless farming, data collection - big data - decision support systems, e-commerce and agricultural marketing, imaging - image processing, agricultural robots - autonomous devices and unmanned aerial vehicles, precision agriculture and geographical information systems It is extremely essential to carry out studies on smart irrigation - spraying - fertilization and farm animal software through multidisciplinary projects.

It is necessary to conduct comprehensive studies on quality as well as quantity on the sustainability of both the environment and human health, focusing on more conscious use of natural resources for the production of wheat and dry bean plants, which are important for sustainable agriculture and food security, and on researching the usage areas of healthier products to be evaluated in different industries.

REFERENCES

Aktaş, H., Baloch, F., Özberk, F., Oral, E., Doğan, S., Kahraman, M., & Çiğ, F. (2018). Türkiye'nin Güneydoğu Anadolu bölgesinin buğday genetik kaynakları bakımından potansiyeli ve sürdürülebilir olarak korunması. *Journal of Bahri Dagdas Crop Research, 7(2)*, 47–54.

Akter, N., & Rafiqul Islam, M. (2017). Heat stress effects and management in wheat: A review. *Agronomy for Sustainable Development*, 37.

Altieri, M. A. (2018). Agroecology: The Science of Sustainable Agriculture. *Boca Raton*: CRC Press.

Anonymous. (2021). Tarım Ürünleri Piyasları Buğday Haziran-2021. Retrieved from: https://arastirma.tarimorman.gov.tr/tepge.

Anonymous. (2011). United Nations. World Population to reach 10 billion by 2100 if Fertility in all Countries Converges to Replacement Level. World Population Prospects: The 2010 Revision, Press Release. Retrieved from: http://esa.un.org/wpp/Other-Information/Press_Release_WPP2010.pdf

Anonymous. (1990). Cereals. PBI Cambridge, *Plant Breeding International*. Cambridge.

Anonymous. (2019). Sürdürülebilir Toprak Yönetimi Gönüllü Kılavuz İlkeleri. Food and Agriculture Organization. Retrieved from: http://www.fao.org/3/i6874tr/I6874TR.pdf

Avican, Ö. (2019). Fasulye (*Phaseolus vulgaris* L.) ıslah programında genitör olarak kullanılabilecek genotiplerin ve bazı ticari çeşitlerin SSR ve SNP analizleri kullanılarak genetik karakterizasyonu. Namık Kemal Üniversitesi, *Fen Bilimleri Enstitüsü*, Tekirdağ.

Aydemir, L. Y., & Yemenicioğlu, A. (2013). Potential of Turkish Kabuli type chickpea and green and red lentil cultivars as source of soy and animal origin functional protein alternatives. *LWT-Food Science and Technology, 50(2),* 686–694. https://doi.org/10.1016/j.lwt.2012.07.023

Aydınşakir, K., Büyüktaş, D., DİnÇ, N., & Karaca, C. (2015). Impact of salinity stress on growing, seedling development and water consumption of peanut (*Arachis hypogaea* cv. NC-7). *Journal of Akdeniz University Agriculture Faculty, 28(2),* 77–84.

Aysu, A. (2018). Buğday: Beslenme Kültürü ve Politikalar. *Meltem, İzmir Akdeniz Akademisi Dergisi*, *4*, 82-90.

Azarpazhooh, E., & Boye, J. I. (2013). Composition of Processed Dry Beans and Pulses. In M. Siddiq, M. A. Uebersax (Eds.), Dry Beans and Pulses Production, Processing and Nutrition. First edition, *John Wiley & Sons*, pp. 103-1224.

Azeez, M.A., Adubi, A.O., Durodola, F.A. (2018). Landraces and Crop Genetic Improvement. In Rediscovery of Landraces as a Resource for the Future. IntechOpen, http://dx.doi.org/10.5772/intechopen.75944.

Aznar-Sancheza J. A., M. Piquer-Rodriguezb, F. J. Velasco-Munoza & F. Manzano-Agugliarod. (2019). Worldwide research trends on sustainable land use in agriculture. Land Use Policy, 87, 104069. https://doi.org/10.1016/j.landusepol.2019.104069

Bagdi, A., Toth, B., Lorincz, R., Szendi, S., Gere, A., Kokai, Z., Sipos, L., & Tömösközi, S. (2016). Effect of aleurone-rich flour on composition, baking, textural, and sensory properties of bread. LWT - *Food Sci. and Tech*., 65, s762-769.

Balfourier, F., Bouchet, S., Robert, S., De Oliveira, R., Rimbert, H., Kitt, J., Choulet, F. et al. (2019). Worldwide phylogeography and history of wheat genetic diversity. *Sci. Adv*., 5, eaav0536.

Baloch, F. S., Alsaleh, A., & Andeden, E. E. (2016). High levels of segregation distortion in the molecular linkage map of bread wheat representing the West Asia and North Africa region. *Turkish Journal of Agriculture and Forestry*, 40, 352–364.

Banach, J. K., Majewska, K., & Zuk-Gołaszewska, K. (2021). Effect of cultivation system on quality changes in durum wheat grain and flour produced in North-Eastern Europe. Plos one, 16(1), e0236617.

Barut, H., Aykanat, S., Haklı Heybet, E., Eker, S., & Çakmak, İ. (2019). Topraktan ve Yapraktan Azot ve Çinko Uygulamalarının Buğdayda Tane Çinko ve Demir Konsantrasyonları Üzerine Etkisi. *Toprak Su Dergisi,* 2019, s:130-139.

Berk, A. (2016). Türkiye'de Kuru Fasulye Üreten İşletmelerin Ekonomik Analizi ve Etkinliklerinin Belirlenmesi, Çukurova Üniversitesi, *Fen Bilimleri Enstitüsü*, Doktora Tezi, 113s, Adana.

Betancur-Ancona, D., Sosa-Espinoza, T., Ruiz-Ruiz, J., Segura-Campos, M., & Chel-Guerrero, L. (2014). Enzymatic hydrolysis of hard-to-cook bean (*Phaseolus vulgaris* L.) protein concentrates and its effects on biological and functional properties. I*nternational Journal of Food Science and Technology,* 49, 2–8.

Bhat, Z. F., Morton, J. D., Bekhit, A. E. A., Kumar, S., & Bhat, H. F. (2021). Emerging processing technologies for improved digestibility of muscle proteins. *Trends in Food Science and Technology,* 110, s226–239.

Bilişli, A. (2012). Gıda Teknolojisi. Sidas Yayınları, Ankara, 235 s.

Boye, J., Zare, F., & Pletch, A. (2010). Pulse proteins: processing, characterization, functional properties and applications in food and feed. Food Research International, 43, pp 414–431.

Brankovic, G., Dodig, D., Pajic, V., Kandic, V., Knezevic, D., Djuric, N., & Zivanovic, T. (2018). Genetic parameters of T*riticum aestivum* and *Triticum durum* for technological quality properties in Serbia. Zemdirbyste, 105(1), 39-48s.

Braun, H.J., Atlin, G. and Payne, T. (2010). Multi-location testing as a tool to identify plant response to global climate change. In: Reynolds, M.P. (Ed.), Climate Change and Crop Production. CABI, Wallingford, UK, pp. 115–138.

BUGEM. (2022). Kuru Fasulye Bülteni, Bitkisel Üretim Genel Müdürlüğü Tarla ve Bahçe Bitkileri Daire Başkanlığı, Ocak 2022, 18. [Retrieved from: https://www.tarimorman.gov.tr/BUGEM/Belgeler/B%C3%BCltenler/OCAK%202022/Kuru%20Fasulye%20Ocak%20B%C3%BClteni.pdf]

Bulut, S. (2012). Ekmeklik buğdayda kalite. Erciyes Üniversitesi Fen Bilimleri Enstitüsü, *Fen Bilimleri Dergisi, 28(5*), 441-446.

Cakmak I, Pfeiffer WH, McClafferty B 2010. Biofortification of durum wheat with zinc and iron. *Cereal Chem* 87, 10–20.

Camberato, J., & Casteel, S. (2017). Sulfur deficiency. Purdue Univ. Dep. of Agronomy, Soil Fertility Update.

Carbonaro, M., Maselli, P., & Nucara, A. (2015). Structural aspects of legume proteins and nutraceutical properties. *Food Research International*, 76, 19–30.

Celmeli, T., Sari, H., Canci, H., Sari, D., Adak, A., Eker, T., & Toker, C. (2018). The nutritional content of common bean (*Phaseolus vulgaris* L.) landraces in comparison to modern varieties. *Agronomy*, *8(9*), 166.

Chahal, H. S., Sing, A., & Malhi, G. S. (2020). Role of Sulphur nutrition in oilseed crop production-A review. *Journal of Oilseed Brassica*, *11(2*), 95-102.

Chavez-Mendoza, C., Hernandez-Figueroa, K.I., & Sanchez, E. (2019). Antioxidant capacity and phytonutrient content in the seed coat and cotyledon of common beans (*Phaseolus vulgaris* L.) from various regions in Mexico. *Antioxidants, 8(1*), 5.

Chen, P. X., Tang, Y., Marcone, M. F., Pauls, P. K., et al. (2015). Characterization of free, conjugated, and bound phenolics and lipophilic antioxidants in regular-and non-darkening cranberry beans (*Phaseolus vulgaris* L.). *Food Chem*., 185, s298–308.

Ciudad-Mulero M, Matallana-Gonzalez MC, Callejo MJ, Carrillo JM, Morales P, Fernandez-Ruiz V (2021). Durum and bread wheat flours. Preliminary mineral characterization and its potential health claims. *Agronomy*, *11(1*), 108.

Cowling, W.A. (2013). Sustainable plant breeding. *Plant Breed*, 132, 1–9.

Cömert, M., Gün, A. (2020). 'Fonksiyonel gıda olarak mor ekmek'. *Journal of International Social Research*, 12(74).

Çadraku H.S. (2021). Groundwater quality assessment for irrigation: case study in the Blinaja River Basin, Kosovo. *Civil Engineering Journal, 7(9),* 1515–1528.

Çeker, A. (2016). Sürdürülebilir Tarım ve Türkiye Açısından Bir Değerlendirme. International Periodical for the Languages, Literature and History of Turkish or Turkic, 11(2), 809-836. DOI: http://dx.doi.org/10.7827/TurkishStudies.9496, ISSN: 1308-2140.

Damodaran, S. (2006). Protein: Denaturation, In Y.H. Hui (Eds.). Handbook of food science, technology, and engineering. CRC Press Taylor & Francis, pp. 6.

Daryanto, S., Wang, L., Jacinthe, P.-A. (2016). Global Synthesis of Drought Effects on Maize and Wheat Production. *PLOS ONE, 11(5),* e0156362.

Day, L. (2013). Proteins from land plants - Potential resources for human nutrition and food security. *Trends in Food Science and Technology, 32(1),* 25–42.

Delcour, J.A., & Hoseney, R.C. (2010). Principles of cereal science and technology, 3rd ed. AACCI International, Inc., St. Paul, MN.

Demir, M. K., Bilgiçli, N., Türker, S., & Demir, B. (2019). Farklı Stabilizasyon İşlemleri Uygulanmış Buğday Ruşeymlerinin Depolama Özellikleri. *Necmettin Erbakan Üniversitesi Fen ve Mühendislik Bilimleri Dergisi, 1(2),* 67-75.

Dibaba, D.G. (2015). The impact of improved haricot bean variety on income among farming households in Southern Ethiopia. *International Journal of Multidisciplinary Advanced Research Trends, 2(3)*, 10–19.

Dinu, M., Whittaker, A., Pagliai, G., Benedettelli, S., & Sofi, F. (2018). Ancient wheat species and human health: Biochemical and clinical implications. *Journal of Nutritional Biochemistry,* 52, 1–9.

DSI. (2019). Toprak ve su kaynakları. Retrieved from: http://www.dsi.gov.tr/toprak-ve-su-kaynaklari

Duru, S., Gül, A., & Hayran, S. (2019). Türkiye'de Buğday ve Buğday Mamulleri Dış Ticaret Yapısı. *Bilecik Şeyh Edebali Üniversitesi Sosyal Bilimler Dergisi, 4(2*), 552-564. DOI: 10.33905/bseusbed.563347

Dziki, D., & Laskowski, J. (2005). The factors that influence the energy requirements of the grinding process of wheat grain. TEKA Komisji Motoryzacji i Energetyki Rolnictwa, 5, 55-64.

El, S. N. (2016). Gıda bileşenlerinin beslenme açısından önemi. Ege Üniversitesi, Mühendislik Fakültesi, Öğrenciler İçin Hazırlanmış Ders Notu, s54, 56.

Eliasson, J. (2015). The rising pressure of global water shortages. *Nature, 517(7532),* 6.

Erekul, O. (2017). Tarla Bitkileri Bölümü Lisans Ders Notları, Aydın Adnan Menderes Üniversitesi Ziraat Fakültesi, Aydın.

Erekul, O., & Yiğit, A. (2018). Buğdayda Tane Dolum Dönemindeki Yüksek Sıcaklığın Protein Yapısına Etkisi. Uluslararası Tarım, Çevre ve Sağlık Kongresi, Aydın.

Ertaş, N. (2013). Baklagil Ve Baklagil Ürünlerinin Gıda Endüstrisinde Kullanımı. Ulusal Kop Bölgesel Kalkınma Sempozyumu, 14-16 Kasım 2013, Konya.

FAO. (2016). Pulses, nutritious seeds for sustainable future. A journey through all regions of the planet Brazil, China, India, Mexico, Morocco, Pakistan, Spain, Tanzania, Turkey, USA and recipes from some of the most prestigious chefs in the World.

FAO. (2005). Global Network on Integrated Soil Management for Sustainable use of Salt-affected Soils. Food and Agricultural Organization of the United Nations, Rome.

FAO. (2021). Cereal Supply and Demand Brief. Retrieved from: http://www.fao.org/world-foodsituation/csdb/en/

FAO. (2014). Retrieved from: http://www.fao.org/worldfoodsituation/csdb/en/

FAO. (2020). Sustainable Development Goals. Retrieved from: https://www.fao.org/sustainable-development-goals/goals/goal-2/en/

Garg, G., & Kumari, R. (2016). Functional analysis of plants SRG-genes/transmembrane protein (RLKs) under stress condition. *Journal of Bioscience and Agriculture Research, 9(02)*, 827-836.

Gasmalla, M.A.A., Tessema, H.A., Salaheldin, A., Alahmad, K., Hassanin, H.A.M., Aboshora, W. (2017). Health Benefits of Milk and Functional Dairy Products. *MOJ Food Process Technology, 4(4),* 108-111.

Göymen, M. A. (2022). BBM Fırıncılık, Bisküvi ve Makarna Sektörü İhtisas Dergisi, Sayı 56, (Eylül-2022), pp. 56-61.

Gupta, A. K., Maheshwari, A., & Khanam, R. (2020). Assessment of phosphorus fixing capacity in different soil orders of India. *Journal of Plant Nutrition, 43(15),* 2395-2401.

Güçbilmez Ç M, Şahin M, Akçacık A G, Aydoğan S, Demir B, Hamzaoğlu S, et al. (2019). Evaluation of GlutoPeak Test for Prediction of Bread Wheat Flour Quality, Rheological Properties and Baking Performance. *Journal of Cereal Science*, 90, 1-9.

Gülümser, A. (2016). Dünyada ve Türkiye'de Yemeklik Dane Baklagillerin Durumu. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 25(1),* 292–292. https://doi.org/10.21566/tarbitderg.280511

Güvercin, D. (2019). Mucize Buğdayda (*Triticum turgidum* var. *mirabile*) Başak ve Çiçek Meristem Kimlik Genlerinin Klonlanması ve Karakterizasyonu. Doktora Tezi, Süleyman Demirel Üniversitesi, *Fen Bilimleri Enstitüsü*, Biyoloji Anabilim Dalı, Isparta. 109s.

Haliloğlu, K., Türkoğlu, A., Öztürk, H.I., Özkan, G., Elkoca, E., & Poczai, P. (2022). Retrotransposon Markers in the Analysis of Genetic Diversity among Common Bean (*Phaseolus vulgaris* L.), Germplasm from Türkiye. Genes (Basel), 13(7), 1147.

Hasanuzzaman, M., Nahar, K., & Fujita, M. (2013). Plant Response to Salt Stress and Role of Exogenous Protectants to Mitigate Salt-Induced Damages.

Hegab, O.W., Abdel-Latif, E.F., Zaki, H.M.B.A., & Moawad, A.A. (2021). Fundamental Role of Lactobacillus plantarum and Inulin in Improving Safety and Quality of Karish Cheese. *Open Veterinary Journal, 11(3),* 356-363.

Houria B., Mahdi K., & Zohra T.F. (2020). Hydrochemical Characterisation of Groundwater Quality: Merdja Plain (Tebessa Town, Algeria). *Civil Engineering Journal, 6(2),* 318–325.

Huen J, Börsmann J, Matullat I, Böhm L, Stukenborg F, Heitmann M, et al. (2018). Wheat Flour Quality Evaluation from the 'Baker's Perspective: Comparative Assessment of 18 Analytical Methods. *European Food Research and Technology, 244(3),* 535–545.

Jankielsohn, A. & Miles, C. (2017). How do Older Wheat Cultivars Compare to Modern Wheat Cultivars Currently on the Market in South Africa? *Journal Hortic Sci Res, 1(2),* 42-47.

Jarvan, M., Edesi, L., Adamson, A., Lukme, L., &Akk, A. (2008). The Effect of Sulphur Fertilization on Yield, Quality of Protein and Baking Properties of Winter Wheat. *Agronomy Research, 6(2),* 459-469.

Kang, J.S., Singh, H., Singh, G., & Kang, H. (2017). Abiotic Stress and Its Amelioration in Cereals and Pulses: A Review. *International Journal of Current Microbiology and Applied Sciences, 6(3),* 1019–1045.

Karaduman, Y., Belen, S., Olgun, M., Ceyhan, M., Kartal, Y., & Özkan, K. (2020). Ülkesel ekmeklik buğday alım bareminde ekmeklik buğday çeşitlerinin ayrımı: yapay zeka destekli örüntü tanıma uygulamalarının kullanılabilirliği. *Biyoloji Bilimleri Araştırma Dergisi, 13(2),* 45–59.

Kaya, B. (2009). İklim değişikliğinin Türkiye'de buğday, arpa ve mısır bitkilerinin verimleri üzerine etkilerinin panel veri modeli ile analizi. (Yüksek Lisans Tezi) İstanbul Üniversitesi, *Sosyal Bilimler Enstitüsü*, İstanbul.

KB. (2014). 10. Kalkınma Planı. Kalkınma Bakanlığı, Ankara.

Kılıç, H. (2009). Azot ve fosfor uygulamalarının Şahin-91 arpa (Hordeum vulgare L.) çeşidinde klorofil içeriğine etkilerinin belirlenmesi. *Türkiye VIII. Tarla Bitkileri Kongresi* -Poster Bildiriler, 535- 539.

Kızılgeçi, F., Yıldırım, M., Albayrak, Ö., Başdemir, F., & Akıncı, C. (2016). Farklı Azot Dozlarının Makarnalık Buğday F2 Melez Kombinasyonlarında Bazı Kalite Parametreleri Üzerine Etkisi. *Dicle Üniversitesi Fen Bilimleri Enstitü Dergisi*, 2016, 5(1), 7–14.

Kızmaz, M. Z., & Gümüş, M. (2021). Yemeklik Baklagil Tohumlarıyla Taşınan Bazı Virüslerin Tespiti, Biyolojik ve Moleküler Karakterizasyonu. *Türk Tarım ve Doğa Bilimleri Dergisi, 8(3),* 784–799.

Kiszonas, A. M., & Morris, C. F. (2018). Wheat breeding for quality: a historical review. *Cereal Chemistry, 95(1),* 17–34.

Köksel, H., Çetiner, B., & Şanal, T. (2016). Hububat ürünleri konusunda yanıltıcı iddialar ve bilimsel değerlendirmeler. *TUSAF Dergisi*, 1, 50–66.

Lence B., Moosavian N., & Daliri H. (2017). Fuzzy programming approach for multiobjective optimization of water distribution systems. *Journal of Water Resources Planning and Management*, ASCE 143(7), 04017020.

Lin, D., Lu, W., Kelly, A.L., Zhang, L., Zheng, B., & Miao, S. (2017). Interactions of vegetable proteins with other polymers: Structure-function relationships and applications in the food industry. *Trends in Food Science & Technology,* 68, 130–144.

Lv A., Qi S., & Wang G. (2022). Multi-model driven by diverse precipitation datasets increases confidence in identifying dominant factors for runoff change in a subbasin of the Qaidam Basin of China. *Scince Total Environment*, 802, 149831. https://doi.org/10.1016/j.scitotenv.2021.149831.

Mackay, I., Horwell, A., Garner, J., White, J., McKee, J., & Philpott, H. (2011). Reanalyses of the historical series of UK variety trials to quantify the contributions of genetic and environmental factors to trends and variability in yield over time. *Theor Appl Genet, 122(1)*, 225–238. https://doi.org/10.1007/s00122-010-1438-y.

Marschner, H. (2011). Marschner's mineral nutrition of higher plants. Academic Press.

Mathew, I., Shimelis, H., Mutema, M., Clulow, A., Zengeni, R., Mbava, N., & Chaplot, V. (2019). Genome-wide association study of drought tolerance and biomass allocation in wheat. *PLoS One*, *14(12*), e0225383.

Matus, I. A., & Hayes, P. M. (2002). Genetic diversity in three groups of barley germplasm assessed by simple sequence repeats. *Genome*, 45, 1095–1106.

Menteş Yılmaz, Ö. (2011). Türkiye'de yetiştirilen başlıca buğday çeşitlerinin antioksidan aktivitelerinin ve fenolik asit dağılımlarının belirlenmesi ve ekmeğin nar kabuğu ekstraktı ile zenginleştirilmesi. Ankara Üniversitesi *Fen Bilimleri Enstitüsü*, Doktora tezi.

Mondal, S., Rutkoski, J. E., Velu, G., Singh, P. K., Crespo Herrera, L. A., Guzman, C., ... Singh, R. P. (2016). Harnessing diversity in wheat to enhance grain yield, climate resilience, disease and insect pest resistance and nutrition through conventional and modern breeding approaches. *Frontiers in Plant Science*, 7, 991.

Morales, S. G., Trejo-tellez, L. I., Carlos, F., Merino, G., Espinosa-victoria, D., Edgar, B., & Cabrera, H. (2012). Growth, photosynthetic activity, and potassium and sodium concentration in rice plants under salt stress. *Acta Scientiarum, 34(3),* 317–324.

Morgounov A., Özdemir F., Keser M., Akın B., Payne T., Braun H. J. (2019). International Winter Wheat Improvement Program: History, Activities, Impact And Future. Front. *Agr. Sci. Eng., 6(3*), 240–250.

Mudgil, D. (2017). The Interaction Between Insoluble and Soluble Fiber. In R. A. Samaan (Ed), Dietary Fiber for the Prevention of Cardiovascular Disease (35-59). Academic Press. https://doi.org/10.1016/B978-0-12-805130-6.00003-3.

Mutlu, A., & Taş, T. (2020). Türkiye'de yetiştirilen bazı ekmeklik buğday çeşitlerinin yarı kurak iklim koşullarında (*T. aestivum* l.) kalite özellikleri ile verim ve verim unsurlarının incelenmesi. *Avrupa Bilim ve Teknoloji Dergisi*, (19), s344–353.

Nachimuthu, V. V., Muthurajan, R., Duraialaguraja, S., Sivakami, R., Pandian, B. A., Ponniah, G., ... Sabariappan R. (2015). Analysis of population structure and genetic diversity in rice germplasm using SSR markers: An initiative towards association mapping of agronomic traits in Oryza sativa. *Rice*, 8-30.

Nawaz, F., Yasin Ashraf, M., Ahmad, R., & Waraich, E. A. (2013). Selenium (Se) seed priming induced growth and biochemical changes in wheat under water deficit conditions. *Biological Trace Element Research, 151(2),* 284-293.

Nellemann, C., Corcoran, E., Duarte, C. M., Valdés, L., De Young, C., Fonseca, L., & Grimsditch, G. (2009). Blue Carbon. A Rapid Response Assessment. United Nations Environment Programme, GRID-Arendal. [http://www.grida.no/publications/rr/blue-carbon/]

Önder, M., Ateş, K. M., Kahraman, A., & Ceyhan, E. (2012). "Konya İlinde Fasulye Tarımında Karşılaşılan Problemler Ve Çözüm Öneriler." *Tarım Bilimleri Araştırma Dergisi, 5(1),* 143-148. Issn: 1308-3945, E-Issn: 1308- 027, Konya.

Özberk, F., Karagöz, A., Özberk, İ., & Atlı, A. (2016). Buğday genetik kaynaklarından yerel ve kültür çeşitlerine; Türkiye'de buğday ve ekmek. *Tarla Bitkileri Merkez Araştırma Enstitüsü Dergisi, 25(2),* 218–233.

Özdemir, E., Sade, B., Soylu, S., & Atalay, E. (2012). "Ekmeklik buğday (*Triticum aestivum* L.)'da priming uygulamalarının kurak ve normal ortam koşullarında büyüme parametreleri ile bağıl su içeriği değerleri üzerine etkileri." *Selçuk Tarım ve Gıda Bilimleri Dergisi, 26(2),* 25–30.

Özkan, R., Özberk, İ., Özberk, F., & Bayhan, M. (2018). "Buğdayda azota tepki ve karlı çeşit tercihi." *Dicle Üniversitesi Fen Bilimleri Enstitüsü Dergisi, 7(2),* 50-60.

Paximada, P., Howarth, M., & Dubey, B. N. (2021). "Double Emulsions Fortified with Plant and Milk Proteins as Fat Replacers in Cheese." *Journal of Food Engineering,* 288, 110229, 1-8.

Pehlivan, A., Ünver İkincikarakaya, S., Kaplan Evlice, A., Şanal, T., Salantur, A., & Yazar, S. (2018). "Makarnalık buğday genotipleri ile yabani makarnalık buğdaylar arasındaki melez hatlarının (F5, F6) kalite özelliklerinin değerlendirilmesi." Türkiye Yerel Buğdaylar Sempozyumu, 20-22 Aralık 2018, Bolu, Özet kitapçığı, Sayfa 55-56 (Sözlü sunum).

Peterson, C. J., Graybosch, R. A., Baenziger, P. S., & Grombacher, A. W. (1992). "Genotype and environment effects on quality characteristics of hard red winter wheat." *Crop Science,* 32, 98-103.

Pfeiffer, W. H., & McClafferty, B. (2007). "Biofortification: breeding micronutrient-dense crops." In: Kang MS, Priyadarshan PM (eds) Breeding major food staples. *Blackwell Science,* New York, pp 61–91.

Raffan, S., Oddy, J., & Halford, N. G. (2020). "The sulphur response in wheat grain and its implications for acrylamide formation and food safety." *International Journal of Molecular Sciences, 21(11*), 3876.

Rahmati, N. F., Koocheki, A., Varidi, M., & Kadkhodaee, R. (2018). "Introducing Speckled sugar bean (*Phaseolus vulgaris*) protein isolates as a new source of emulsifying agent." *Food Hydrocolloids*, 79, 498–508.

Ranum, P. M., Barrett, F. F., Zoewe, R. J., & Kulp, K. (1990). "Nutrient levels in internationally milled wheat flours." *Cereal Chemistry*, 51, 361.

Rebello, C. J., Greenway, F. L., & Finley, J. W. (2014). "Whole grains and pulses: A comparison of the nutritional and health benefits." *Journal of Agricultural and Food Chemistry, 62(29)*, 7029-7049.

Rengel Z, Batten GD, Crowley DE 1999. "Agronomic approaches for improving the micronutrient density in edible portions of field crops." *Field Crop Research*, 60, 27–40.

Reyes-Moreno, C., & Paredes-López, O. (1993). "Hard-to-cook phenomenon in common beans." Critical Reviews in Food Science and Nutrition, 33, 227-286.

Ricci, L., Umilta, E., Righetti, M. C., Messina, T., Zurlini, C., Montanari, A., … Bertoldo, M. (2018). "On the thermal behavior of protein isolated from different legumes investigated by DSC and TGA." *Journal of the Science of Food and Agriculture, 98(14)*, 5368–5377.

Ryan, L., Thondre, P. S., & Henry, C. J. K. (2011). "Oat-based breakfast cereals are a rich source of polyphenols and high in antioxidant potential." J*ournal of Food Composition and Analysis*, 24, 929–934.

Sansaloni, C., Franco, J., Santos, B., Percival Alwyn, L., Singh, S., Petroli, C., Dreher, K., Payne, T., Marshall, D., +19 more. (2020). "Diversity analysis of 80,000 wheat accessions reveals consequences and opportunities of selection footprints." *Nature Communications*, 11, 4572.

Sarıoğlu, G., & Velioğlu, Y. S. (2018). "Baklagillerin Bileşimi." Akademik Gıda, 16(4), 483–496. https://doi.org/10.24323/akademik-gida.505547

Sarto, M. V. M., Sarto, J. R. W., Rampim, L., Rosset, J. S., Bassegio, D., da Costa, P. F., & Inagaki, A. M. (2017). "Wheat phenology and yield under drought: a review." *Australian Journal of Crop Science, 11(8),* 941–946.

Scaglia, B., Nunes, R. R., Rezende, M. O. O., Tambone, F., & Adani, F. (2016). "Investigating organic molecules responsible for auxin-like activity of humic acid fraction extracted from vermicompost." *Science of the Total Environment*, 562, 289-295.

Seddon, N. H., J. H. Xiaoting, Pye, T., Reid, H., Roe, D., Mountain, D., & Rizvi, A. R. (2016). "Ecosystem-Based Adaptation: A Win-Win Formula For Sustainability in A Warming World?" Briefing July 2016, IUCN.

Sepehri, A., & Sarrafzadeh, M. H. (2018). "Effect of nitrifiers community on fouling mitigation and nitrification efficiency in a membrane bioreactor." *Chemical Engineering and Processing,* 128, 10–18. https://doi.org/10.1016/j.cep.2018.04.006.

Sevilmiş, G. (2013). "Yükselen trend: Fonksiyonel gıdalar." Ar&Ge Bülten, 39.

Sezgin, C. A., & Bülbül, S. (2017). "Türk sanatı ve mutfak kültüründe buğday." *Uluslararası Sosyal Araştırmalar Dergisi, 10(54),* 1080–1091.

Sharma, A., Sharma, G. D., Singh, Y., Sharma, M., Katoch, V., & Sharma, K. C. (2013). "Optimum sowing dates and varieties for seed productivity of pole Frenchbean (*Phaseolus vulgaris* L.) under north western Himalayas." *African Journal of Agricultural Research, 8(48),* 6196-6201.

Sharma, I., Tyagi, B. S., Singh, G., Venkatesh, K., & Gupta, O. P. (2015). "Enhancing wheat production- a global perspective." *Indian Journal of Agricultural Sciences*, 85, 3–13.

Sheaffer, C. C., & Moncada, K. M. (2012). "Crop Profiles: Grasses-Wheat Introduction to Agronomy Food, Crops and Environment 2nd Edition (vice president editorial: Dave GARZA), pp. 541-547, USA."

Shekari, F., Abbasi, A., & Mustafavi, S. H. (2017). "Effect of silicon and selenium on enzymatic changes and productivity of dill in saline condition." *Journal of the Saudi Society of Agricultural Sciences, 16(4),* 367–374.

Shewry, P. R., & Hey, S. (2015). "Do 'ancient' wheat species differ from modern bread wheat in their contents of bioactive components?" *Journal of Cereal Science,* 65, 236-243.

Shi, Z., Yao, Y., Zhu, Y., & Ren, G. (2016). "Nutritional composition and antioxidant activity of twenty mung bean cultivars in China." *Crop Journal, 4(5),* 398–406.

Shiferaw, B., Smale, M., Braun, H., Duveiller, E., Reynolds, M., & Muricho, G. (2013). "Crops that feed the world 10. Past successes and future challenges to the role played by wheat in global food security." *Food Security, 5(3),* 291–317.

Singh, U., Praharaj, C. S., Singh, S. S., Bohra, A., & Shivay, Y. S. (2015). "Biofortification of pulses: Strategies and challenges." In Proceedings of The Second International Conference on Bio-Resource and Stress Management, Hyderabad, India, pp: 50-55.

Sonkurt, M. (2018). "Bitki Gelişimini Teşvik Edici Bakteri (PGPB) Uygulamalarının Ekmeklik ve Makarnalık Buğdayda Gelişme, Verim ve Verim Öğeleri Üzerine Etkisinin Belirlenmesi." Siirt Üniversitesi, *Fen Bilimleri Enstitüsü,* Yüksek Lisans Tezi, 74s, Siirt.

Souza, E. J., Martin, J. M., Guttieri, M. J., O'Brien, K. M., Habernicht, D. K., Lanning, S. P., McLean, R., Carlson, G. R., & Talbert, L. E. (2004). "Influence of Genotype, Environment, and Nitrogen Management on Spring Wheat Quality." *Crop Science*, 44, 425-432.

Sun, M., Zhan, M., Zhao, M., Tang, I. I., Qin, M. G., Cao, C. G., Cai, M. L., Jiang, Y., & Liu, Z. H. (2019). "Maize and Rice Double Cropping Benefits Carbon Footprint and Soil Carbon Budget In Paddy Field." *Field Crops Research*, 243, 107620.

Süzer, S. (2021). Sürdürülebilir Tarım (Tarımda Verimliliği Arttırmanın ve Sürdürülebilir Tarımın Esasları). Trakya Tarımsal Araştırma Enstitüsü Müdürlüğü, Edirne/Türkiye. https://arastirma.tarimorman.gov.tr/ttae/Sayfalar/Detay.aspx?SayfaId=72.

Şanlıer, N. (2013). "Tam tahıllar ve sağlık = Ekmek." *Standart Ekonomik ve Teknik Dergi*, 70-75.

Tabtabaei, S., Konakbayeva, D., Rajabzadeh, A. R., & Legge, R. L. (2019). "Functional properties of navy bean (*Phaseolus vulgaris*) protein concentrates obtained by pneumatic tribo-electrostatic separation." *Food Chemistry*, 283, 101–110.

TAGEM. (2021). Gübre Sektör Politikası 2018-2022. Retrieved from: https://www.tarimorman.gov.tr/TAGEM/Belgeler/yayin/G%C3%BCbre%20Sekt%C3%B6r%20Politika%20Belgesi%202018-2022.pdf

TASAV. (2022). "İklim Krizinin Tarım, Gıda Sektörü ile Su Kaynaklarına Etkisi." Küresel İklim Değişikliği ve Sosyo-Ekonomik Etkileri Kitabı (85-116). Retrieved from: https://www.tasav.org/media/k2/attachments/Kitap_K%C3%BCresel_%C4%B0klim_De%C4%9Fi%C5%9Fikli%C4%9Fi.pdf

The World Bank. (2019). Annual freshwater withdrawals, agriculture. Retrieved from: http://data.worldbank.org/indicator?display=graph

TMO. (2018). Hububat Sektör Raporu. Retrieved from: http://www.tmo.gov.tr/Main.aspx?ID=40

Toklu, F., & Yağbasanlar, T. (2005). "Ekmekli buğdayda (*Triticum aestivum* L.) bitki boyu, başaklanma süresi, bayrak yaprak alanı ve tane ağırlığının kalıtımı üzerine bir araştırma." Türkiye VI. Tarla Bitkileri Kongresi, 2, 689-694.

Tsoukala, A., Papalamprou, E., Makri, E., Doxastakis, G., & Braudo, E. E. (2006). "Adsorption at the air-water interface and emulsification properties of grain legume protein derivatives from pea and broad bean." Colloids and Surfaces B: Biointerfaces, 53(2), 203–208.

TUIK (2022). TUIK İstatistik Veri Tabanı. Retrieved from: https://biruni.tuik.gov.tr/medas/?kn=92&locale=tr. Türkiye İstatistik Kurumu, Çankaya, Ankara, Türkiye.

TUIK. (2020). Retrieved from: http://www.tuik.gov.tr/PreTablo.do?alt_id=1001

Ugvuanyi, S., Udengwu, O.S., Snowdon, R.J., & Obermeier, C. (2022). "Novel candidate loci for morpho-agronomic and seed quality traits detected by targeted genotyping-by sequencing in common bean." *Frontiers in Plant Science*, 13, 1-18.

UHK (2011). Buğday Raporu. Ulusal Hububat Konseyi. Ankara, 69 s.

USDA (2022). United States Department of Agriculture, Foreign Agricultural Service. World Agricultural Production. Retrieved from: https://apps.fas.usda.gov/psdonline/circulars/production.pdf

Wakchaure, G.C., Minhas, P. S., Ratnakumar, P., & Choudhary, R. L. (2016). "Optimising supplemental irrigation for wheat (*Triticum aestivum* L) and the impact of plant bio-regulators in a semi-arid region of Deccan Plateau in India." *Agricultural Water Management*, 172, 9–17.

Willer, H., Travnicek, J., Meier, C., & Schlatter, B. (2022). The World of Organic Agriculture Statistics and Emerging Trends 2022. Research Institute of Organic Agriculture FiBL, IFOAM – Organics International, 341 pp.

Wu, H., Guo, S., Guo, P., Shan, B., & Zhank, Y. (2021). "Agricultural Water and Land Resources Allocation Considering Carbon Sink/Source and Water Scarcity/Degradation Footprint." *Science of the Total Environment*, 819, 152058.

WWF (2016). Türkiye'nin Buğday Atlası. Retrieved from: http://awsassets.wwftr.panda.org/downloads/turkiye_nin_buday_atlas_web.pdf

WWF (2021). Türkiye'de Tarım Topraklarının Dünü, Bugünü ve Geleceği. WWF-Türkiye (Doğal Hayatı Koruma Vakfı), İstanbul, Türkiye, 2021.

WWF (World Wildlife Fund.) (2013). Operational Framework For Ecosystem-Based Adaptation Implementing And Mainstreaming Ecosystem-Based Adaptation Responses In The Greater Mekong Sub-Region. The World Bank Group, Washington, DC 20433, USA.

Yadav, A. N., Kour, D., Kaur, T., Devi, R., & Yadav, N. (2020). "Agriculturally important fungi for crop productivity: current research and future challenges." In Agriculturally important fungi for sustainable agriculture, 275-286.

Yazar, A., & Kaya, C.I. (2014). "A New crop for salt affected and dry agricultural areas of Turkey: quinoa (*Chenopodium quinoa* Willd.)." *Turkish Journal of Agricultural and Natural Sciences, 2 (2014),* 1440–1446.

Zaharieva, M., & Monneveux, P. (2014). "Cultivated einkorn wheat (*Triticum monococcum* L. subsp. *monococcum*): the long life of a founder crop of agriculture." *Genet Resour Crop Evol*., 61, 677–706.

Zeleke, K. T., & Nendel, C. (2016). "Analysis of options for increasing wheat (*Triticum aestivum* L.) yield in south-eastern Australia: The role of irrigation, cultivar choice and time of sowing." *Agricultural Water Management*, 166, 139-148.

Zhang, H., Tan, S. N., Wong W. S., Ng, C. Y. L., Teo, C. H., Ge, L., Chen X., & Yong, J.W.H. (2014). "Mass spectrometric evidence for the occurrence of plantgrowth promoting cytokinins in vermicompost tea." *Biology and Fertility of Soils, 50(2),* 401-403.

Zhang, J., Zhang, S., Cheng, M., Jiang, H., Zhang, X., Peng, C., Lu, X., Zhang, M., & Jin, J. (2018). "Effect of Drought on Agronomic Traits of Rice and Wheat: A Meta-Analysis." *International Journal of Environmental Research and Public Health, 15 (5),* 839.

Zheng, X., Shang, J., Yue, Q., & Li, M. (2019). "Wheat and Wheat Hybrids." In Bioactive Factors and Processing Technology for Cereal Foods. Springer, Singapore, pp. 77-102.

ZMO (2019). Ülkemizde Gübre Üretimi ve Tüketimi Üzerine Değerlendirme. Retrieved from: https://www.zmo.org.tr/genel/bizden_detay.php?kod=30873&tipi=5&sube=3

Zou, M., Kang, S., Niu, J., & Lu, H. (2020). "Untangling the effects of future climate change and human activity on evapotranspiration in the Heihe agricultural region, Northwest China." *Journal of Hydrology*, 585, 124323.

Zucaro, F., & R. Morosini (2018). "Sustainable land use and climate adaptation: a review of European local plans." Tema. *Journal of Land Use, Mobility and Environment, 11 (1),* 7-26.

CHAPTER 2

TÜRKİYE's NATIVE PLANT SPECIES *Aquilegia olympica*[1]

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INTRODUCTION

Türkiye with different climates and geomorphological structure is one of the world's most important gene center. *Aquilegia olympica* belongs to the rich biodiversity of the country and has high commercial potential as ornamental plants with nice flowers (Figures 1 and 2). *Aquilegia olympica* from family Ranunculaceae is commonly called columbine and it is a clump-forming perennial that typically grows on branched upright stems.

It has been known as *Aquilegia vulgaris* var. Olympica and *Aquilegia caucasica* in the past (https://www.ukwildflowers.com/).

It is naturally grown in Turkey, Iran, Georgia, Armenia, Azerbaijan and spreads in the Northern Caucasus. Amasya, Artvin, Erzincan, Gümüşhane, Kayseri, Kahramanmaraş, Van and Trabzon have the natural distribution of *Aquilegia olympica* in Turkey. It is a herbaceous perennial plant with 30-60 cm stems and attractive flowers. Flowering time of this native species is in the spring and summer months. It is named ‘Haseki küpesi’ in Turkish in Türkiye means Haseki earring.

The population of *Aquilegia olympica* in Türkiye has been decreasing due to different kinds of threats. While there are some studies in the world to save their population and to commercialize this species, there is almost no study yet in our country to protect, propagate, cultivate and introduce this species to the ornamental sector.

Aquilegia olympica has potential of usage in landscaping as outdoor plant and potted plant indoor with plant height control. In this review, some information about *Aquilegia olympica* species and its natural distribution in Turkey, as well as some research studies were given and reviewed in order take attention and emphasize the importance of propagating, cultivating and protecting this species in Türkiye.

1. *Aquilegia olympica* in BOTANY

Aquilegia spp. from the *Ranunculaceae* family. Species belonging to the genus are perennial, herbaceous plants with rhizomes. *Aquilegia* spp., which is mostly distributed in temperate regions. Aquilegia (*Ranunculaceae*), commonly known as Columbine, has been used in ecological and breeding studies for more than 50 years (Hodges et al., 2002).

Aquilegia is a member of the *Ranunculaceae* family in the eudicot order. With this nuclear arrangement it is sister to the rest of the dicotyledons. Aquilegia therefore constitutes an important third data point in deep evolutionary comparisons between dicotyledons and monocotyledonous plants such as Arabidopsis, Petunia and Antirrhinum (Kramer, 2009). This transferability between species gives Aquilegia a great advantage as a model system (Yang et al., 2005).

2. *Aquilegia olympica in the WORLD*

Aquilegia 76 species are grown in Eurasia, North America and parts of Central America (Robert, 2003). It has 17 taxa (species, subspecies and hybrids) in Asia (Erst et al., 2013). It is mostly found in northern temperate and mountainous regions. Many hybrids exist and a few varieties are sold commercially as ornamental plants. In addition, Aquilegia species occupy a wide range of habitats, from coastal areas to dry rocky areas and from low to high altitudes. *Aquilegia* species are among the valuable flowers used to decorate gardens in spring and early summer (Robert, 2003). They have been cultivated for centuries due to the diversity in flower morphology and color. The plants bloom showy flowers in many shades of purple, blue, lavender, red, pink, yellow or white (Yang et al., 2005).

3. *Aquilegia olympica in* TÜRKİYE

Türkiye is one of the countries with a very rich flora in the world, with more than 12.000 plant species, which is almost the same number of plants in the European continental flora, with higher endemism ratio, around 30% are endemic to country (Güner et al., 2000). Among this flora richness, *Aquilegia* L. is represented by only one species (*Aquilegia olympica*) in Türkiye (Figures 1 and 2).

4. BIOLOGY of *Aquilegia olympica*

The flowers of *Aquilegia olympica* are bell-shaped and white-blue in color (Figures 1 and 2). It is a perennial herbaceous plant that grows 30–60 cm tall and has showy flowers. The stem of the basal leaves is divided into three small stems, and each has three leaflets at the end. The leaflets are lobed and round-toothed, the upper side of the leaves is hairless, the lower side is felty and softly hairy (Güleryüz and Vural, 2009).

Flowering time is between June and July. The flower head is bent forward. The sepals are purplish blue in color and measure 25–35 mm. White petals are colored purplish blue. It is generally seen in moist grassland areas where spruce forests are located, at an altitude of 1700–2800 m (Güleryüz and Vural, 2009). *Aquilegia olympica* is diploid and has n = 7 chromosomes (Clark, 2009).

5. DISTRUBUTION of *Aquilegia olympica* in TÜRKİYE

Distribution areas of *Aquilegia olympica* according to some research studies conducted in Türkiye are as follows:

- ✧ Damal, Pasof district of Kars, Arap Tombs-Derindere Village, forest areas, 1300-2500 m (Demirkuş and Erik, 1994)
- ✧ Artvin, Karagöl. In the flora of Sahara National Park (Artvin) and its surroundings, at 1910 m (Eminağaoğlu and Anşin, 2004)
- ✧ Artvin, Borçka, Yıldız Lake and around Efeler village, at 1910 m (Eminağaoğlu et al., 2008)
- ✧ Artvin, Borçka, Atanoğlu Village, Suvazvare Hill, subalpine vegetation, humid areas, at 1901 m (Eminağaoğlu et al., 2007)
- ✧ vegetation of Artvin-Atila (Hatilla) Valley National Park between 1900 and 2400 m altitude (Anşin, 1984)
- ✧ Artvin urban forest (Tilki et al., 2008)
- ✧ Trabzon Maçka district Altındere Valley 1900 m, Taşköprü 1680 m and around Çevlik, Düzköy, around Haçkalı Baba plateau and Maçka Hamsiköy (Uzun and Anşin, 2006)
- ✧ Gümüşhane province, Giresun-Dereli Kuzalan in the nature park (Koday et al., 2018)
- ✧ Ardahan, Posof, on the way to Al village, field edges, at 1550 m (Esen, 2010)
- ✧ Giresun; Espiye District, Karadoğan Town, on Sümbüllübük road, roadside between 1715-1790 m (Şenel et al., 2014)
- ✧ Erzincan, Çayırlı, Keşiş Mountain, coastal parts of Aksu stream and Çayırlı, Keşiş Mountain, Üzümlü Plateau, Çamlık locality, next to Damlayan Stone and its surroundings, 2300 m (Korkmaz, 2008)

- ✧ Isparta Kirişli Mountain Tırtar village, at 975 m (Selvi and Yıldırım, 2004)
- ✧ Yozgat-Tokat provinces and geographically in the Deveci Mountains (Bingöl et al., 2010)
- ✧ Kayseri province In the flora of Mount Erciyes within the borders, in the east of Sakar farm, at 1700-2200 m (Vural and Aytaç, 2005)
- ✧ North of Kahramanmaraş, Göksun, Kınıkkoz village, in the P. nigra forest, at 1500 m and in Bitlis (Yıldız, 2001)
- ✧ Kalem Mountain, Doğanlı village, 1800 m, in open forests (Altıok and Behçet, 2005).

Figure 1: Flower of *Aquilegia olympica* plants naturally grown in Trabzon province (Ö Sarı, Trabzon, Düzköy, Haçkalı Baba yaylası)

Figure 2: *Aquilegia olympica* plants naturally grown in Artvin province (FG Çelikel, Artvin 2012)

Flower of *Aquilegia olympica* plants naturally grown in Trabzon province (Ö Sarı, Trabzon, Düzköy, Haçkalı Baba yaylası) is shown in Figure 1. Native *Aquilegia olympica* plants naturally grown in Artvin province (FG Çelikel, Artvin 2012) is shown in Figure 2. Distribution of the *Aquilegia olympica* over Turkey based on provinces (Amasya, Artvin, Erzincan, Gümüşhane, Kayseri, Kahramanmaraş, Van) is given in Figure 3 according to the Turkish Plants Data Service 'TÜBİVES' (2023, Version 2.0 BETA).

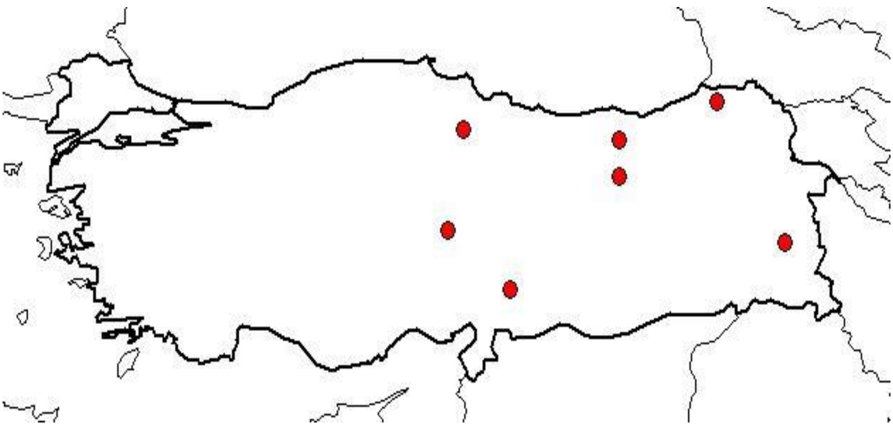

Figure 3: Distribution of the *Aquilegia olympica* Over Turkey. Based on Vilayets: Amasya, Artvin, Erzincan, Gümüşhane, Kayseri, Kahramanmaraş, Van. Turkish Plants Data Service 'TÜBİVES' (2023) Version 2.0 BETA.

6. MEDICINAL and ORNAMENTAL USAGES

As a medicinal and aromatic plant, *Aquilegia olympica* stems have been used in folk medicine (in Trabzon, Black Sea region of Türkiye) to treat constipation. Additionally, it has diuretic and diaphoretic effects (Üçüncü et al., 2009). Compounds of leaves, inflorescences, vernalization and longevity are topics worth investigating (Kramer and Hodges, 2010). It is known that *Aquilegia olympica* plant has a medicinal value.

Aquilegia olympica is used for landscaping purposes in parks and gardens in the world with its showy atractive flowers with nice colors and shapes. It has also potential plant that can be used as potted plants indoor and cut flowers in vase with its long stems and beautiful flowers, as long as it has a satisfactory vase life which should be studied.

7. 'HASEKİ EARRING' in IUCN RED LIST CLASSES

Red List Classes and Criteria (Figure 4) of the International Union for Conservation of Nature and Natural Resources (IUCN) are designed to classify species with high risks extinction (Allen et al., 2014).

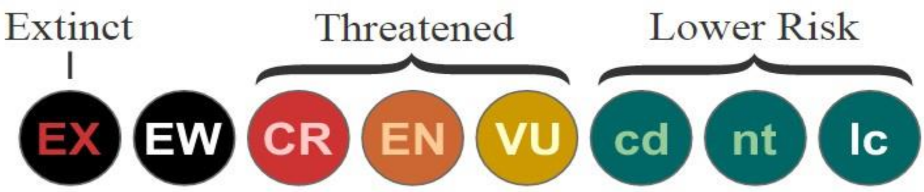

Figure 4: IUCN (International Union for Conservation of Nature & Natural Resources) Risk Categories (Allen et al., 2014)

In Türkiye, in the areas where *Aquilegia olympica* spreads naturally, decreases in population density occur due to construction, human activities, tourism activities, grazing and agricultural activities. Despite these threats, *Aquilegia olympica* is included in the not evaluated (NE) category on the IUCN list.

8. SUGGESTIONS and CONCLUSION

Aquilegia species are used as an ornamental plant around the world with its showy and impressive flowers. The species are very important as ornamental plants and medicinally. Many varieties of *Aquilegia* species have been developed around the world and are sold commercially as potted ornamental plants and seeds. These species are also used as cut flowers but the usage as cut flowers is limited although it has a potential.

Although many studies have been conducted on the species of *Aquilegia* species in the world, there is almost no research studies on *Aquilegia olympica* distributed in Türkiye as well as its use as an ornamental plants.

In Türkiye, many of our natural plants are in danger of extinction due to reasons such as intense construction in the spreading areas of natural plants, human activities, tourism activities, grazing and collecting. Apart from the efforts to protect our natural plants, many of which are endemic and therefore their disappearance from the country would lead to extinction in the world, cultivation and propagation efforts should be accelerated by developing the most appropriate methods (Çelikel, 2015; 2023).

In ornamental plant breeding all over the world, in addition to cultivar development, identifying and introducing new cultivars and species that have not yet been brought into production has gained importance, and it has become mandatory to benefit from flora with the same characteristics in the development of outdoor plants, especially for climates with subtropical conditions such as our country (Kostak, 1998).

It is important to benefit from the natural vegetation of the regions in the arrangements made to meet recreational needs. This not only makes the work effective in terms of aesthetics and functionality, but also ensures the integration of the regions with their immediate surroundings. In addition, not having any adaptation problems reduces maintenance and facility costs (Topay and Kaya, 1998). For these reasons, the use of natural plants for landscaping purposes has become widespread in recent years, especially in European countries.

As a result, it is recommended to increase the use of natural plants in landscape areas instead of imported ones. It is necessary to reduce the share of imported plants, which are unfortunately currently widely used in landscaping, and to use this resource in developing domestic production. This will ensure that the country's dependence on foreign sources in this regard is eliminated and that native plant resources are utilized. 'Haseki Küpesi' (earring) is an important species waiting to be evaluated for cultivation and breeding studies both purposes as medicinal and ornamental plants in Türkiye.

REFERENCES

Allen, D., Bilz, M., Leaman, D.J., Miller, R.M., Timoshyna, A., Window, J. (2014). European Red list of medicinal plants, http://www.iucnredlist.org/initiatives/europe/publications

Altıok, A., Behçet, L. (2005). The flora of Bitlis River Valley. *Turkish Journal of Botany*, 29(5): 355-387.

Anşin, R., Özkan, Z. C., Eminağaoğlu, Ö. (1984). Artvin-Atila (Hatilla) Vadisi Milli Parkinin Vejetasyon Yapisina Genel Bir Bakis. *Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi*, 1(1): 59-71.

Bingöl, M. Ü., Ketenoğlu, O., Geven, F., Güney, K. (2010). Deveci Dağları (Yozgat-Tokat) Vejetasyon Tiplerinin Floristik Kompozisyonu Üzerine Bir Araştırma. *Biyoloji Bilimleri Araştırma Dergisi*, 1:65-71.

Clark, M. J. (2009). Characterization and breeding of *Aquilegia* (Ranunculaceae) and Malva (Malvaceae) species for drought tolerance and aesthetics (Doctoral dissertation, University of Guelph).

Cullen, J., Heywood, V. H., & Akeroyd, J. R. (1964). *Aquilegia* L. *Flora Europaea*, 1: 238-240.

Çelikel, F.G. (2015). Süs bitkilerinde tohumluk (tohum, fide, fidan, soğan) üretimi ve kullanımı. *SÜSBİR Dergisi*, 3: 32-33.

Çelikel, F.G. (2023). Lecture notes on ornamental plants. Ondokuz Mayıs University, Department of Horticulture, Samsun.

Demirkuş, N., Erik, S. (1994). Çiçek Dağı (Ulgar) ve Çevresinin (Posof/Kars) Florası Üzerine Bir Araştırma. *Hacettepe Fen ve Mühendislik Bilimleri Dergisi*, 15:1-47.

Eminağaoğlu, Ö., Anşin, R. (2004). Flora of the Karagöl-Sahara National Park (Artvin) and Its Environs. *Turkish Journal of Botany*, *28*(6), 557-590.

Eminağaoğlu, Ö., Kutbay, H. G., Özkan, Z. C., ve Ergül, A. (2008). Flora of the Camili Biosphere Reserve Area (Borçka, Artvin, Turkey). *Turkish Journal of Botany*, 32(1), 43-90.

Eminağaoğlu, Ö., Yüksek, T., Gümüş, S., Kurdoğlu, O., Eraydın, S. (2007). *Karagöl (Borçka-Artvin) Tabiat Parkı ve Yakın Çevresinin Flora ve Vejetasyonu*, TÜBİTAK .

Erst, A.S., Vaulin, O.V. (2013). Phylogenetic Relationships Among North Asian species of the genus Aquilegia based on molecular markers. *Russian Journal of Genetics: Applied Research*, 4(1): 35-42.

Esen, S. (2010). Ardahan İli, Posof, Damal ve Hanak İlçeleri Florası. Yüksek Lisans Tezi, Fen Bilimleri Enstitüsü, Artvin Çoruh Üniversitesi, 226s.

Güleryüz, S.A.G., Vural, C.O. 2009. Türkiye'nin 120 Alpin bitkisi, *OBANET*, 96s.

Güner, A., Özhatay, N., Ekim, T., Başer, K.H.C. (2000). Flora of Turkey, Volume 11, Edinburgh University Press. Edinburgh.

Hodges, S.A., Whittall, J.B., Fulton, M., Yang, J.Y.(2002). Genetics of Foral Traits İnfluencing Reproductive İsolation Between *Aquilegia formosa* and *Aquilegia pubescens. Am. Nat.* 159 (Suppl 3), pp:51-60.

Koday, S., Kaymaz, H., Kaya, G. (2018). Kuzalan Tabiat Parki'nin Doğa Turizm Potansiyeli (Dereli-Giresun). *Marmara Coğrafya Dergisi*, 37:124-143.

Korkmaz, M. (2015). *Floristical Diversity and Endemic Plants of Çayırlı District (Erzincan/Turkey*). 8/3: 223-247.

Kostak, S. (1998).Türkiye Florasında Doğal Olarak Bulunan Süs Bitkilerinin Kullanımı, Değerlendirmesi ve Muhafazası. Türkiye I. Ulusal Süs Bitkileri Kongresi, Yalova, pp:6-9.

Kramer, E. M. (2009). Aquilegia: A New Model For Plant Development, Ecology, and Evolution. Annu. Rev. *Plant Biology*., 60: 261-277.

Kramer, E.M., Hodges, S.A. (2010). Aquilegia As a Model System for the Evolution and Ecology of Petals, Philosophical Transactions of the Royal Society of London B: *Biological Sciences*, 365: 477-490.

Robert, N. (2003). Columbines: *Aquilegia, Paraaquilegia*, and *Semiaquilegia*, Timber Press, Portland, pp: 90-91.

Selvi, B., Yıldırım, Ş. (2004). B3 (Isparta) Karesi için Yeni Bitki kayıtları. *Selçuk Üniversitesi Fen Fakültesi Fen Dergisi*, *1*(23), 61-67.

Şenel, F., Başköse, İ., Tuğ, G. N. (2014). Contributions to The Flora of the Region Between Alucra-Espiye-Yağlidere (Giresun/Turkey) Within The Prepontic Zone. *Biological Diversity and Conservation*, 7(3): 58-73.

Tilki, F., Güner, S., Tüfekçioğlu, A. (2008). Kent Ormancılığı ve Artvin İli Kent Ormancılığı Uygulamaları. *Artvin Çoruh Üniversitesi Orman Fakültesi Dergisi*, 9 (1-2): 92-100.

Topay, M., Kaya, Z. (1998). Bartın-Amasra Florasındaki Bazı Otsu Süs Bitkilerinin Peyzaj Değerlerinin Saptanması ve Kentsel Mekanlarda Kullanım Olanakları. I. Ulusal Süs Bitkileri Kongresi Tebliği, Yalova, pp:74-82.

TÜBİVES, (2023). Türkiye Plants Data Service-Taxa in Provinces. http://www.tubives.com

Uzun, S. P., Anşin R. (2006). Subalpine and Alpine Flora of Altındere Valley (Maçka, Trabzon). *Turkish Journal of Botany*, 30(5): 381-398.

Üçüncü, O., Yayli, N., Volga, C., Yayli, N., Terzioglu, S. (2009). Chemical Composition of the Essential Oils From the Flower, Leaf and Stem of *Aquilegia olympica* Grown in Turkey, *Asian Journal of Chemistry*, 21(8): 6569.

Vural, C., Aytaç, Z. (2005). The Flora of Erciyes Dağı (Kayseri, Turkey). *Turkish Journal of Botany*, 29(3): 185-236.

Yang, J. Y., Counterman, B. A., Eckert, C. G., Hodges, S.A. (2005). Cross-Species Amplification of Microsatellite Loci in Aquilegia and Semiaquilegia (*Ranunculaceae*). *Mol. Ecol. Notes*, 5: 317–320.

Yildiz, B. (2001). Floristical Characteristics of Berit Dağı (Kahramanmaraş). *Turkish Journal of Botany*, 25(2): 63-102.

CHAPTER 3

IMPACTS OF ALIEN WEED INVASION ON AGRICULTURAL PRODUCTION AND BIODIVERSITY IN PAKISTAN

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INTRODUCTION

It is projected that weeds will cost the world economy $400 billion a year (Oerke et al., 1994). According to estimates, invasive plants cost the United States alone $34.7 billion a year (Pimentel et al., 2000). According to Oerke's calculations, 34% of potential crop losses globally are caused by weeds, while 16% and 18% are caused by diseases and animal pests, respectively (Oerke, 2006). Over the past ten years, weeds-or more broadly, invasive plants have gained recognition for their detrimental effects on a wide range of human endeavors beyond agriculture, including forestry, transportation, human health, recreation, and tourism (Pimentel et al., 2000; EEA, 2012). Historically, the agricultural sector has borne the majority of the costs associated with weed management. Even though it is difficult to evaluate these combined economic consequences precisely, the estimates that have been provided indicate that these are risks that need to be carefully taken into account. When taken as a whole, these economic impacts need quick attention, particularly as weeds present a dynamic threat and react differently to different management approaches (Clements et al., 2004; Clements and DiTommaso 2011).

In Pakistan, like in many other parts of the globe, invasive alien weeds may have a considerable influence on agricultural output and biodiversity. These invasive plants are non-native species that may displace native plants and disturb regional ecosystems when they are introduced to a new habitat. Pakistan has suffered severe sociological, ecological, and economic losses as a result of invasive weeds (Shah et al., 2022). Due to elements including temperature adaptability, human activity, and natural dispersion processes, invasive weeds in Pakistan have the potential to spread throughout a variety of places (Khan et al., 2013).

In agricultural productivity, invasive weeds are the primary cause of direct financial losses. With a substantial ecological impact, one of the primary direct causes of environmental change on a worldwide scale is biological invasion. Global agriculture, which continues to have an impact on food security globally, may be significantly impacted by invasive alien plant species (Fleming et al., 2021). The cost of plant invasion to agriculture is rising due to the increasing number of new introductions, which have a big impact on crop yield. Invasive weed distribution and potential spread are influenced by a number of important variables, including:

Climate Suitability: Invasive weeds often flourish in conditions that are similar to those found in their natural habitats. A variety of invasive species may grow and spread in Pakistan due to its varied environment, which ranges from dry to temperate.

Human Activities: Trade, travel, agriculture, and urban expansion are examples of human activities that may unintentionally import and spread invasive plants. Invasive plant seeds and propagules may travel via ports, transportation systems, and agricultural practices.

Natural Dispersal: Invasive plants may spread by means of the wind, water, and animals in a natural way. Some plants generate vast numbers of thin, wind-transportable seeds that may travel great distances. Seeds and plant fragments may travel via water, particularly when aquatic invasive species are present.

Disturbed Habitats: Agricultural fields, building sites, and locations hit by natural catastrophes are examples of disturbed habitats where invasive plants often flourish. These disruptions give invasive species a chance to grow and displace native vegetation.

Poor Soil and Water Management: Poor soil and water management techniques may lead to an environment that is ideal for the development and proliferation of invasive weeds. Invasive plants may spread more easily due to improper irrigation, soil erosion, and water management.

Ecological Resilience: Invasive plant colonization may be more likely to affect ecosystems that have been modified by causes like deforestation, urbanization, or pollution. Weaknesses in these changed ecosystems may be taken advantage of by invasive plants. When native species are unavailable or unable to compete successfully in new habitats, invasive weeds may fill these ecological niches.

Examples of invasive plant species that might grow and proliferate in Pakistan include:

In Pakistan, a number of invasive weed species have been found. These plants were either inadvertently or purposefully imported since they are not indigenous to the area. Invasive weeds in Pakistan include, for instance:

1) *Cassia tora* (Sicklepod): Due to its competition with crops for resources, the invasive plant *Cassia tora* may have an adverse effect on agriculture fields. Additionally, the quality of harvested crops may be harmed by their seeds.

2) *Parthenium hysterophorus*: Parthenium weed, sometimes known as Congress Grass, is one of Pakistan's most infamous invasive weeds. It may induce allergic responses in people and cattle and competes with natural plants for resources. It has a detrimental effect on biodiversity and agricultural output. Due to its resilience to many climatic conditions and quick development, it is already common in many areas of Pakistan and has the potential to expand much further (Khan et al., 2013; 2014)

3) *Prosopis juliflora* (Mesquite): Originally planted for its wood and shade, mesquite has spread aggressively and has a severe effect on natural ecosystems by competing with native species and lowering water availability.

4) *Lantana camara* (also known as lantana) is a very invasive shrub that can grow quickly and create thickets, displacing other plant life. It may harm the ecosystems of forests and grazing pastures. Lantana is currently widespread across Pakistan, and because birds and other animals spread its seeds, it has the potential to spread to new locations.

5) *Ageratum conyzoides*: *Ageratum conyzoides*, sometimes known as Billygoat species. This weed, particularly in disturbed landscapes and agricultural fields, may flourish in a variety of environments and may spread to new places. It has the ability to grow into thick stands, outcompeting other vegetation and upsetting local plant communities.

6) *Mikania micrantha*: *Mikania micrantha* sometimes known as the Mile-a-Minute Weed, is a quick-growing vine that may cover native plants and block sunlight. Along the boundaries of forests and in disturbed regions it is especially hazardous.

7) *Eichhornia crassipes:* *Eichhornia crassipes* also known as Water hyacinth, is predominantly an aquatic plant, but it may rapidly blanket water bodies, making them difficult to navigate, irrigate, and support biodiversity. It impacts aquatic creatures and lowers the oxygen content of the water (Fawad et al., 2013).

8) ***Chromolaena odorata*** (Siam Weed): is a potential invader in disturbed places and along forest boundaries due to its wind-dispersed seeds and quick development. Siam weed, is an invasive plant that may take over damaged regions and harm natural ecosystems and biodiversity. Additionally, it releases substances that stop other plants from growing.

9) ***Datura stramonium*** (Jimson Weed): This poisonous plant may overtake fields and rangelands, endangering both cattle and crops.

10) ***Eupatorium adenophorum*** (Crofton Weed): The invasive shrub known as Crofton Weed creates dense thickets and limits the amount of fodder available to animals. Native plant communities are also impacted.

The effects of these invasive weed species on agriculture, natural ecosystems, and public health varies. Agriculture, ecosystems in general, human health, and regional economy are all impacted by these losses. Here are some main categories of losses brought on by invasive weeds in Pakistan, while precise quantification of these costs may change across time and place.

1. Agricultural Losses:

Reduced crop yields: Because invasive weeds compete with crops for sunshine, nutrients, and water, crop yields are reduced, costing farmers money. Some invasive weeds may contaminate harvested crops, lowering their quality and market value.

Increased management costs: Herbicides, labor, and other management and control strategies are expensive for farmers and agricultural businesses.

2. Losses to Biodiversity and Ecosystems

Disruption of native plant communities: Invasive weeds have the ability to change the structure and composition of native plant communities, which reduces biodiversity and ecosystem services.

Habitat degradation: Invasive weeds may weaken native animal and plant habitats by lowering favorable circumstances.

Loss of native species: Invasive weeds may outcompete native plants, causing native species populations to fall and perhaps even become extinct locally.

3. Losses to Human Health and Livestock:

Allergic responses: Certain invasive weeds, such as Parthenium weed, may induce allergic responses in people, which can result in health problems and higher medical expenses (Khan et al., 2013).

Toxicity to livestock: Some invasive weeds are hazardous to livestock when eaten, endangering both the welfare of the animals and the viability of agricultural operations.

4. Economic Effects:

Decreased land value: The presence of invasive weeds may result in a decline in the value of the land, which may have an impact on property values and restrict viable land uses.

Changes in soil composition: Some invasive weeds have the capacity to influence the fertility of the soil by changing the soil's composition and nutrient cycle. The capacity of native plants and crops to grow may be affected by this.

Tourism and leisure: By destroying natural landscapes and restricting outdoor activities, invasive weeds may have a detrimental effect on tourism and leisure.

5. Resource and Water Losses:

Water body degradation: Water bodies may become clogged by invasive aquatic plants like water hyacinth, which can also lower water quality and make it difficult to navigate and irrigate.

Increased water consumption: Some invasive plants have a higher water need than native species, which exacerbates the problem of water shortage.

6. **Costs of management and oversight:**

Costs of control measures: Communities and government organizations spend money on labor, equipment, and herbicides to manage and control invasive weed infestations.

7. **Opportunity costs:**

Lost Productivity: Land and resources taken up by invasive weeds may have been put to better use, earning cash and perhaps even helping the neighbourhood.

8. **Costs of resilience and adaptation:**

Reduced ecosystem resilience: Invasive weeds may weaken ecosystems' resistance to natural disasters like droughts, floods, and disease outbreaks, necessitating greater resources for recovery.

POSSIBLE MANAGEMENT INTERVENTIONS FOR INVASIVE WEEDS

A personalized and adaptable strategy to control is crucial given the wide variety of invasive species and environments. The objective is usually to lessen the effect of invasive species and restore environmental balance rather than full eradication. In order to effectively manage invasive species and safeguard native biodiversity, ecosystem health, and human well-being, a variety of regulatory measures, public involvement, scientific research, and strategic interventions are needed.

Pakistan has implemented a comprehensive approach that encompasses many strategies such as preventative measures, early detection methods, integrated management practices, public awareness campaigns, and international collaboration to address the entry and proliferation of invasive species. It is crucial that Pakistan implements methods that prioritize speedy addressing the detection, prevention, and effective management of alien invasive weed species in order to limit these consequences. This would allow Pakistan to better manage the situation.

Khan et al. (2020) suggest that a variety of approaches might be used in order to slow the spread of invasive species once they have already been introduced. The deployment of integrated pest management (IMW) methods, public education efforts and awareness campaigns, the construction of monitoring and surveillance systems, and the promotion of worldwide cooperation are the measures that are included in this approach to the problem.

The major goal of weed management is to avoid the initial introduction of invasive species, rapidly identify their existence, apply effective control methods, and offset any negative consequences that may be caused by these species. It is necessary for varied stakeholders, including farmers, academic organizations, government departments, and local communities, to work together in order for initiatives to be successfully implemented, regardless of whether the efforts are proactive or reactive in approaches.

The following are few fundamental strategies for managing invasive weeds:

1. Prevention:

Monitoring and quarantine: To stop the dissemination of invasive species, strict rules on the import and transportation of plants and plant products are needed.

Public awareness and education: To prevent the spread of invasive species, the import and export of plants and plant products must be tightly controlled.

2. Early Detection and Quick Action:

Monitoring and surveillance: To identify new infestations early, regularly check for the presence of invasive species.

Early alert systems: Set up procedures for informing the appropriate authorities of any new invasive species occurrences so that swift action may be taken.

3. Utilizing Integrated Management:

Mechanical Control: Employ proactive methods such as hand pulling, trimming, and mowing to eradicate unwanted plants.

Chemical control: Use herbicides sparingly and in compliance with advised application protocols to target and manage invasive plants while minimizing harm to non-target species (Khan et al., 2012).

Biological control: To assist in preventing the growth and spread of invasive plants, and introduce bugs or illnesses that are their natural enemies.

Cultural control: Promote native vegetation and restore damaged ecosystems as viable methods of managing land to stop the spread of invasive species.

4. Restoration and Reintroduction of Native Plants:

Plant native species restoration: Plant native species that can outcompete invading ones to help restore environments (Li et al., 2015).

Ecological restoration: Restore natural functions and processes that may aid in the management of invasive species by using ecological principles.

5. Research and observation:

Study: To create efficient control methods, conduct study on the biology, ecology, and management of invasive species.

Monitoring: To make sure that management activities are still successful, continuously check treated areas for regrowth or new infestations.

6. Partnerships & Collaboration:

Government agencies: Create and implement invasive species control policies, rules, and programs.

Community involvement: Involve stakeholders, landowners, and neighborhood communities in invasive species control initiatives.

International Cooperation: Work with neighboring nations and international organizations to stop the spread of invasive species across international borders.

7. Adjustable Management:

Flexibility: Be aware that managing invasive species is a continuous process and that tactics may need to be modified in response to new knowledge and evolving circumstances.

8. Lengthy Commitment:

Sustained Efforts: Acknowledge that managing invasive species needs a long-term commitment and sufficient resources to successfully lessen their effects.

CONCLUSION

In Pakistan, invasive alien weeds are a serious danger to biodiversity, agricultural output, and other aspects of human well-being. These non-native plants have already caused significant harm to the nation's ecology, economy, and society, as this chapter has shown. Natural ecosystems have been upset, agriculture yields have decreased, human health has been jeopardized, and essential resources like water have been stressed. The many effects of invasive weeds need prompt attention as well as coordinated management and control measures. However, eliminating invasive weeds as the only goal is not a simple way to win the war against them. It calls for an all-encompassing, flexible approach that recognizes the complexity of the problem.

RECOMMENDATIONS:

Improved Surveillance and Monitoring: To identify and monitor the spread of invasive weeds, put in place reliable surveillance and monitoring systems. Timely action depends on early detection.

Prevention and Quarantine Measures: To stop the entry of new invasive species into Pakistan, tighten laws and implement stricter quarantine protocols. Strict regulations on the import and transportation of plants and plant products should be part of this.

Public Awareness and Education: Start efforts to educate locals, visitors, and businesses about the dangers posed by invasive species. Promote appropriate behavior to avoid unintentional exposures.

Integrated Management Approaches: Employ integrated management strategies that include biological, chemical, mechanical, and cultural control techniques. Adjust these strategies to the unique traits of every invading species.

Native Species Restoration: Encourage the restoration of native plants so they can outcompete invading weeds. In this sense, ecological restoration initiatives may be beneficial.

Research and Data Collection: Make investments in studies concerning the ecology, biology, and control of invasive species. Encouraging management tactics will be informed by ongoing data gathering and analysis.

Community Engagement: Include stakeholders, landowners, and the local community in programs to manage invasive species. Their enthusiastic involvement may improve the efficiency of management initiatives.

Government Regulations and International Cooperation: Create and implement national laws and policies to fight invasive plants. Furthermore, cooperate with international organizations and neighboring nations to stop the cross-border spread of invasive species.

Flexibility and Long-Term Commitment: Acknowledge that managing invasive species is a continuous process that can call for modifications in the future. Invest consistent time and energy to achieve long-term success.

Adaptation to Climate Change: Take into account the potential effects of climate change on the distribution and behaviour of invasive species. Modify management tactics to take into account shifting environmental circumstances.

Economic Assessments: Comprehensive economic analyses should be carried out in order to determine the exact amount of money that invasive weeds have cost you. Policymakers may more efficiently distribute resources with the use of these analyses.

In conclusion, Pakistan must take proactive, interdisciplinary, and long-term measures to combat the threat posed by invasive alien weeds. By implementing a comprehensive strategy that incorporates prevention, early identification, integrated management, and community participation, Pakistan may lessen the negative effects of these intruders and protect its biodiversity, agricultural output, and general well-being. Since the problems caused by invasive weeds will only become worse with time, the moment to take action is right now.

REFERENCES

Clements D.R, DiTommaso A, Jordan N, Booth B, Murphy S.D, Cardina J et al. (2004). Adapt-ability of plants invading North American cropland. Agric Ecosyst Environ 104:379–398

Clements D.R, DiTommaso A. (2011). Climate change and weed adaptation: can evolution of invasive plants lead to greater range expansion than forecasted? Weed Res 51:227–240

EEA. (2012). The impacts of invasive alien species in Europe. EEA Technical report No 16/2012. European Environment Agency, Copenhagen. doi:10.2800/65864

Fawad, M., Gul, B., Khan, H., & Khan, Z. (2013). Study and collection of hydrophytes of the district Swabi, Pakistan. *Pakistan Journal of Weed Science Research*, *19*(4).

Fleming J. P, Wersal R. M, Madsen J. D, Dibble E. D. (2021). Weak non-linear influences of biotic and abiotic factors on invasive macrophyte occurrence. Aquatic Invasions. 16(2): 349-364.

Khan, H., Khan, M. M., Gul, B., Fawad, M., & Khan, I. (2020). Weeds flora of the Agricultural University Peshawar Research Farm. *Pakistan Journal of Weed Science Research*, *26*(4).

Khan, H., Marwat, K. B., Hassan, G., & Khan, M. A. (2012). Chemical control of *Parthenium hysterophorus* L. at different growth stages in non-cropped area. *Pakistan Journal of Botany*, *44*(5), 1721-1726.

Khan, H., Marwat, K. B., Hassan, G., & Khan, M. A. (2013). Socio-economic impacts of parthenium (*Parthenium hysterophorus* L.) in Peshawar valley, Pakistan. *Pakistan Journal of weed science research*, *19*(3).

Khan, H., Marwat, K. B., Hassan, G., Khan, M. A., & Hashim, S. (2014). Distribution of parthenium weed in Peshawar valley, Khyber Pakhtunkhwa-Pakistan. *Pak. J. Bot*, *46*(1), 81-90.

Khan, H., Marwat, K. B., Khan, M. A., & Hashim, S. (2014). Herbicidal control of parthenium weed in maize. *Pak. J. Bot*, *46*(2), 497-504.

Li W, Luo J, Tian X, et al. (2015). A new strategy for controlling invasive weeds: Selecting valuable native plants to defeat them. Scientific Reports. 2015;5:11004. DOI: 10.1038/srep1100

Oerke E-C, Dehne H.W, Schönbeck F, Weber A. (1994). Crop production and crop protection-estimated losses in major food and cash crops. Elsevier, Amsterdam

Oerke E-C. (2006). Crop losses to pests. J Agric Sci 144:31–43

Pimentel D, Lach L, Ziniga R, Morrison D. (2000). Environmental and economic costs associated with non-indigenous species in the United States. BioScience 50:53–65

Shah, I. A., Khan, H., Gul, B., & Uslu, Ö. S. (2022). Farmers' perception Of Climate Change And PartheniumWeed Distribution In District Swabi, Khyber Pakhtunkhw. *Pakistan Journal of Weed Science Research*, *28*(3).

CHAPTER 4

SUSTAINABLE MANAGEMENT OF PARTHENIUM WEED THROUGH BIOCHAR PRODUCTION

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INTRODUCTION

An essential component of Pakistan's economy is agriculture. It makes up 24% of the GDP. It supports 70% of the rural population's way of life, accounts for 35% of export earnings, and employs 51% of the labor force (Anonymous, 2020). Nevertheless, in order to feed and clothe the crowded millions, increased population pressure requires higher production from agriculture. Conversely, the productive resources are finite and depleting quickly. We anticipate having plenty of healthy food. There are a lot of causes for lower agricultural crop yields, with weed infestation being the most important. Weeds decrease agricultural productivity by competing with crop plants for nutrients, moisture in the soil, and sunshine. According to the majority of weeds are more competitive than agricultural plants. Weed competition is directly correlated with lower agricultural yields. Generally speaking, one kg of crop growth decreases for every kg of weed growth that increases (Rao, 2000).

Significant crop output losses in agriculture are caused by weeds, even with increasing use of agricultural inputs including fertilizers, irrigation, pesticides, and new seed varieties Ameta et al. (2016). The weeds that proliferate in agricultural regions due to the aforementioned inputs compete with crops for nutrients, air, moisture, and light. The yield of many crops is often negatively impacted by weeds, and the total loss in agricultural output is not measured.

Parthenium weed is another dangerous invasive species that affects about 40 countries globally, including Pakistan. Major crops including sugarcane, wheat, and maize are infested by it, and it significantly reduces yields. Out of the approximately 6700 species of weeds that affect agriculture worldwide, 76 have been named the "worst weeds in the world." In Khyber Pakhtunkhwa Province, Marwat (1984) recognized 284 types of weeds. Weeds affect every crop; in wheat, at least 14 weeds have been reported, and in maize and sugarcane, 20 weeds apiece. On farmlands, weeds cause significantly larger annual losses than are actually realized. If one were to translate these losses into monetary terms, the results would be worrisome.

According to studies, Pakistan has yearly crop losses from weeds in the wheat, rice, sugarcane, and cotton crops that range from 17-25%, 20-63%, 10-35%, and 13-31%, respectively (Khan et al., 2014). In Pakistan, there are 54 parasitic weeds, according to Marwat et al. (1993). Around $1010 billion is lost to weeds in agriculture worldwide each year. Australia suffers losses from weeds estimated at $3.3 Billion yearly (Khan et al., 2013), whereas the United States spends over $138 Billion a year controlling invasive plants. According to Khan et al. (2014), weeds in Pakistan's key crops generate losses of around Rs. 130 Billion annually.

Furthermore, invasive plants may be harmful to the health of people and animals (Rai and Singh 2020). Biological invasions have increased over the previous ten years, and over the next twenty years, they are predicted to increase by at least one-third as a result of continuing land-use change, intensification of agriculture, and the development of physical infrastructure. (Seebens et al., 2021). In general, the primary drivers of invasive dispersal dynamics are human disturbances and land degradation. Because they are foreign and invasive, many weeds that are common now did not exist in the wilderness a few years ago. When humans first began cultivating plants for sustenance, they removed unwanted plants from fields, giving rise to the idea of a weed. With the advancement of technology, weed control has become more important (Marwat and Hashim, 2010).

Various invaders or seed importations unintentionally spread weeds. Furthermore, some ecological disruptions, such as disease, fire, land removal, etc. caused changes at both the micro and macro levels and created spaces for new invasive and alien weeds (Marwat et al., 2004). The global floral diversity is in danger from alien and exotic plant invasions, which can have an impact on ecological systems. There is evidence of weed invasion in North-West Pakistan. It was stated that 16 different weeds were invading. *Broussonetia papyrifera, Ailanthus altissima, Pistia stratiotes, Phragmites australis, Parthenium hysterophorus, Cannabis sativa, Galium aparine, Emex spinosus, Amaranthus hybridus, Trianthema portulacastrum, Tagetes minuta, and Imperata cylindrica* were among them (Marwat et al., 2010).

Congress grass and carrot grass are two popular names for *Parthenium hysterophorus* L. Commonly referred to as parthenium weed, *P. hysterophorus* (Asteraceae) is an alien invasive plant that is native to the Neo-tropics and is now found across the pan-tropics. It is an annual or short-lived ephemeral herb. In Australia and India, parthenium weed is regarded as a serious weed. Along roadside ditches, railway tracks, wastelands, degraded areas and rocky nooks, this plant is spreading quickly across Pakistan. It has also been reported to occur in agricultural regions recently. Human health, animal husbandry, agriculture production, and biodiversity are all impacted by this invasive plant (Shabbir and Bajwa, 2007).

P. hysterophorus affects grazing areas, animal health, milk and meat quality, and pasture seed and grain sales in a significant direct and indirect manner. Parthenium weed allergies first manifest as itching, redness, swelling, and blisters around the eyes, cheeks, and neck. Later, the knees and elbows also experience pain. In the latter stages, the skin thickens and darkens. Allergy reactions like dermatitis, asthma, or hay fever may be brought on by pollen, dust, debris, or plant-derived volatile vapors (Shabbir and Bajwa, 2007). It has been shown that twenty parthenium weed plants m^{-2} may reduce maize crop yield in Pakistan by 50% (Rwomushana et al., 1997). Grown at a density of three plants m^{-2}, parthenium weed has been shown by Tamado et al. (2002) to reduce sorghum production by as much as 69% in Ethiopia, where it dropped sorghum grain yield from 40%-97%. Biomass of maize plants was collected by Bajwa et al. (2017) from plots where parthenium weed had been growing for two weeks. This weed's very invasive character might result in a 90% reduction in pasture output in Australia. (Anonymous, 2020).

If parthenium weed is allowed to run wild throughout the growing season, it has been claimed that sorghum grain yields may be reduced by 40-97% (Tamado et al., 2002). Grain filling is reduced by 50% when parthenium weed pollen clusters accumulate on maize floral parts. In addition to competing with farmed crops, parthenium weed also reduces the soil's nutrition supply. Parthenium weed was formerly thought to be an issue on waste and undeveloped areas, but around 1970, reports of its infestation in field crops began to surface. It takes over native natural flora, including therapeutic herbs that humans have long used as a source of medicine, to establish its own territory wherever it invades (Oudhia, 2001).

Its allelopathic qualities seriously endanger biodiversity by suppressing natural plants, including many therapeutic herbs, and preventing germination. Mexico, Central America, and parts of South America are the natural home of parthenium weed. According to Bajwa et al. (2016), parthenium weed reduces agricultural productivity and natural biodiversity while also having a negative impact on animal and human health. The Gujrat area of Punjab province in

Pakistan was the site of the first documented reports of parthenium weed in 1980. It is widely accepted that the weed entered Pakistan from India by commerce and transportation that crossed the Wagah border between the two nations. At the moment, the weed is rapidly taking over the provinces of Azad Jammu & Kashmir, Khyber Pakhtunkhwa (KP), Punjab, and the Islamabad Capital Territory (ICT). Due to widespread irrigated farming, parthenium weed despite being very invasive is believed to have migrated to Punjab's southern districts. It is also believed to have spread to Sind and Baluchistan, two neighboring provinces in Pakistan's south and southwest.

Figure 1 Parthenium weed in infestation on roadsides

P. hysterophorus is an endangered herbaceous plant that thrives in warm climates. It is considered harmful because of its allelopathic qualities (Kohli et al., 2006). As one of the world's ten worst weeds, it is included in the database of invasive species. One mature plant of *P. hysterophorus* may yield between 15,000 and 25,000 seeds throughout the course of its life, making it a prolific weed (Navie et al., 1996). There were still around 200,000 seeds meter^{-2} of contaminated soil. Additionally, its seeds may be able to survive in the soil for up to ten years without any intervention from the plant. After germination, the plant begins to bloom in 4-6 weeks, which helps to produce additional seeds.

Its two distinguishing characteristics very lightweight and non-dormant seeds are what have allowed it to spread to various regions (Ramaswami, 1997). Following the introduction of seeds into both cultivated and uncultivated areas, it spreads quickly, thereby affecting agricultural productivity and biodiversity (Tefera, 2002).

Figure 2 Parthenium weed in infestation in maize crop

Parthenium weed in vegetables

Parthenium weed has infiltrated almost all field crops, pastures, wastelands, yards, fencerows and rights-of-way. It has the potential to diminish agricultural production by 40-97%. According to Khan et al. (2013), parthenium weed caused 30% output losses in sorghum and 20% in maize crops in Pakistan. As a consequence, it may stymie attempts to alleviate poverty, promote economic growth, and ensure food security and long-term development. This plant is found in fodder crops such as maize and sorghum, as well as the Egyptian, Persian, and Swat rangelands, which are among the most important rangelands in the country. We have documented that parthenium weed may infest a variety of grain and fodder crops, as well as vegetables (Khan et al., 2013). Parthenium weed has become the most frequent and densely growing plant in the districts of Swabi, Mardan, and Charsadda, according to Khan et al. (2012).

Figure 3 Parthenium weed infestation in vegetable

Parthenium weed may be used as a soil addition in the form of charcoal to improve soil characteristics. According to Lehmann (2007), biochar is a substance with a high carbon content that is produced when organic material undergoes thermal decomposition at temperatures between 350 and 700 °C while oxygen supply is restricted. It is a large-surface, lightweight, extremely porous substance that may enhance the processes, functions, and qualities of soil (Jeffery et al., 2011). It gives soil bacteria a place to live and has the ability to sequester or store carbon. Through nutrient adhesion to the soil's surface for crop utilization, it improves soil nutrient retention.

The addition of biochar may also improve the soil's cation exchange capacity. Biochar has the potential to retain three times its own weight in moisture due to its increased surface area and pores, which increases its water-holding capacity. Amazonians introduced the use of biochar to boost soil fertility and production. Rich and stable carbon, biochar is a solid that may stay in soil for many years. "Bio" refers to life and comes from "biomass," whereas "char" denotes charcoal. The usage of biochar as a soil supplement sets it apart from charcoal. Biochar is a byproduct of pyrolysis, which is the heat breakdown of organic substances in the absence of air. 2009 (Lehmann and Joseph). Biochar, according to certain hypotheses, may increase soil fertility, provide additional ecosystem services, and trap carbon (C) to help mitigate global warming (Sohi et al., 2010). The major explanations for the observed impacts on soil fertility have been attributed to either enhanced nutrient retention via cation adsorption or a rise in pH in acidic soils (Zwieten et al., 2010). Plant material is pyrolyzed at temperatures between 300 and 1000 degrees Celsius to create biochar, a stable carbon molecule. (Lehmann and Joseph, 2009).

Properties of biochar

Biochar is nearly carbon stable and may persist in the soil for an extended period of time, resulting in decreased greenhouse gas emissions. The agricultural ingredients employed in biomass breakdown alter the properties of biochar. The physical properties of biochar will change and have an influence on how the environment works. This will improve soil aeration and retention of moisture while also increasing the quality and fertility of the soil and attracting soil microbes. The outcomes of increasing crop output via the use of biochar in agriculture are just somewhat positive.

Chemical properties of biochar

Biochar improved the soil pH, lowering its acidity and improving fertilizer and nutrient retention. By combining biochar with other soils, manure, or compost, agricultural soils become more efficient and need less manure to be applied. Because of its nature, biochar prevents nutrients from evaporating. Liquid manures may also be combined with biochar. When biomass waste is slowly pyrolyzed, it produces biochar, a highly porous, fine-grained material with a high concentration of paramagnetic in centres of both organic and inorganic composition. (Lehmann et al., 2006). It has a huge surface area that is made up of oxygen functional groups and aromatic surfaces. (Atkinson et al., 2010).

Characteristics of biochar

It was evident that adding biochar increased the pH of the soil, decreasing its acidity and improving fertiliser and nutrient retention. Farm soils become more productive and need less manure when biochar is mixed with other soils, manure, or compost. Biochar, by its own nature, keeps nutrients from vanishing. Biochar can also be used with liquid manures. Biochar is a fine-grained, highly porous material that is abundant in both organic and inorganic paramagnetic centres. It is primarily used to improve soil and is produced by slowly pyrolyzing biomass waste (Lehmann et al., 2006). According to Atkinson et al. (2010), its enormous surface area is made up of aromatic surfaces and oxygen functional groups.

Effects of biochar in agricultural soil

When soil is treated with biochar, the short-term bio-atmospheric carbon cycle is replaced by the long-term geological carbon cycle, which increases the rate of soil carbon sequestration (Lehmann and associates, 2011). Reviews and studies abound, pointing out that using biochar as a soil supplement has potential advantages beyond carbon sequestration (Sohi et al., 2010). Crops may benefit from improvements to the physical properties of the soil. (Ajayi and Horn, 2016), enhanced soil nutrient availability and retention (Dume et al., 2016), enhanced biological activity through the provision of meta-bolizable organic C substrates (Demisie and Zhang, 2015), translating into higher crop yields (Laghari et al., 2016), and benefits for society through carbon sequestration, which reduces global warming (Zhang et al., 2017).

Soil health

Several studies have shown that adding biochar to soil improves soil health and reduces greenhouse gas emissions. These benefits are especially significant in tropical regions where they help mitigate the effects of drought caused by climate change and enhance soil health. Biochar enhances soil health in both direct and indirect ways. The physical characteristics of soil, such as bulk density, penetration resistance, structure, macro-aggregation, soil stability, pore size distribution, and density, are altered by the addition of biochar; these modifications have a favorable impact on the chemical properties of the soil, such as reduced soil acidity, a reduction in the absorption of soil toxins, increased concentrations of beneficial soil bacteria, and improved cation exchange capacity and nutrient consumption efficiency (Sameera et al., 2021).

Soil microbial activity

For a wide variety of soil microorganisms, biochar offers an appropriate home. Because both the microorganisms and the biochar have a large surface area and are surface hydrophobic, there may be better retention of microbes in soils modified with biochar, which might account for increased activity and variety. Microbes should have a high affinity for biochar since their adherence to solid surfaces rises with surface hydrophobicity. Biochar is a useful tool for enhancing the natural environment and igniting living organisms. Carbonised biomass, such wood ash or charcoal made from rice husks, is a useful ingredient for soil amendments. In addition to increasing the decadal soil carbon pool, the ideal biochar would stimulate the microbial community in soils, resulting in fertilization and the release of nutrients (Venkatesh et al., 2018). According to Tong et al. (2014), applied biochar may shield soil-dwelling microorganisms from natural predators and provide growth environments for them (Thies and Rillig, 2009).

Biochar surface properties and sorption

Despite having the potential to have a net positive or negative surface charge, fresh biochars often have lower initial cation exchange capacities (CEC) than soil organic matter. According to Chan and Xu (2009). A significant interaction with phosphates occurs in certain circumstances, and the first detected anion exchange capacity is remarkable since it eventually disappears in soil (FAO, 2008). When CEC is normalized to surface area, biochar made from high-ash biomass have somewhat higher CEC and charge density. Higher pyrolysis temperatures, on the other hand, result in decreased CEC, especially in charge density, due to the increased surface area created at temperatures as high as 600 ^{0}C and the loss of volatile materials, which may include a large quantity of negative charge and CEC as organic acids. The sorption of more polar organic compounds, such catechol or humic acid extracts, to wood biochars increased in the 400–650 ^{0}C range as a result of the growing nanopore surface area (Kasozi et al., 2010). At temperatures up to 1000 ^{0}C, which are often attained to produce gasification biochars and so-called "activated carbon," carbons are mostly hydrophobic and do not significantly absorb nutrients or polar organic molecules like sugars (Yam et al., 1990).

Biochar physical properties

It is plausible that the physical properties of biochar would impact soil biota in an equivalent manner as its chemical properties. Differences in the physical structure of soils and biochar lead to changes in hydrodynamics, soil tensile strength, and gas movement in a soil biochar combination. These changes should have a significant effect on the soil biota. The degree to which these effects materialize will depend on the feedstock and biochar production conditions, which work together to control the macro- and micro-structure of biochar particles (Downie et al., 2009). Few studies have been conducted to ascertain if these changes are the result of combining two very different materials (biochar and soil) or whether biochar has distinct effects on soil properties at small geographic scales. The addition of biochar may lower the soil's total tensile strength when its tensile strength is lower than that of the soil (such as in clay-rich soils). Therefore, reductions in soil tensile strength may facilitate easier seed germination as well as increase the efficiency of mycorrhizal and root nutrient mining. The bulk density of the soil may also change after applying biochar. (Major et al., 2010).This may affect the soil's water relationships, weed growth, and wildlife.

Biochar Production from Parthenium weed

Congress grass, often referred to as carrot weed, is an invasive plant from which *Parthenium hysterophorus* biochar is extracted. It has many uses in horticulture, agriculture, and environmental management. The allele chemicals present in parthenium weed are removed during the conversion of the biomass into biochar. (Kumar et al., 2013) and improves soil carbon storage and stability (Jeffery et al., 2011). An extremely porous, fine-grained charcoal known as biochar helps soils retain water and nutrients. By decreasing soil acidity, raising crop yields and productivity, and lowering the need for certain chemical and fertilizer inputs, biochar as a soil supplement serves to enhance earth's soil resources (Glaser et al., 2002). Water quality is improved by adding biochar to the soil because it helps the soil hold on to nutrients and agrochemicals for crop and plant uptake. In Lehmann et al. (2003). Pyrolyzing P. hysterophorus (Kumar et al., 2013) is a useful method for producing biochar, which would store carbon and reduce C02 emissions. Evidence of enhanced soil quality after the addition of this biochar includes increased growth of Zea mays, higher basal respiration and microbial biomass carbon, increased activities of catalase and dehydrogenase, decreased soil stress, and improved hydrolytic enzyme activity.

Ambrosin, a parthenium-containing compound with a phototoxic action, was destroyed during charring by high-temperature degradation (Patel, 2011). The soil did not appear to be negatively impacted by the addition of a lot of biochar. The old method of creating biochar for use as a soil additive was by "pit" or "trench" techniques, which resulted in terra preta, or dark soil. This process still has the ability to create biochar in rural regions, but it prevents the harvesting of syngas or bio-oil. Processes for producing biochar may make use of biomass wastes from forestry, agriculture, or urban areas. The goal of pyrolysis is to produce biochar using a modern technology. This may be carried out on a local or big scale. Small-scale production enables subsistence farmers to generate biochar that may be used on their properties or in their gardens. A type of burning known as pyrolysis occurs when heat and an absence of oxygen cause the chemical breakdown of organic molecules. The biochar is produced by burning parthenium biomass for varying periods of time (30-120 minutes) and at varying temperatures (200-500 ◦C). The pyrolysis conditions at this low temperature are suitable for producing stable parthenium biochar (PBC) for soil.

Fe, Mn, Zn, Cu, and Zn are generally more accessible in soils with an acidic pH or higher levels of acidity. (McCauley et al, 2009). However, because biochar can adsorb and immobilise heavy metals, the addition of parthenium biochar to acidic soils in this study reduced micronutrient bioavailability. (Fe, Mn, Cu, and Zn) (Ahmad et al, 2014). Additionally, rates of micronutrients in the soil system dramatically decline when *P. hysterophorus* charcoal doses rise.

Applications for Parthenium Weed Biochar are listed below:

Soil Amendment: To enhance the physical, chemical, and biological qualities of soil, add *Parthenium hysterophorus* biochar to the soil. It promotes nutrient retention, raises the soil's ability to store water, and boosts soil fertility. Plant health and higher agricultural yields may result from this.

Retention of Nutrients: By binding and holding onto nutrients such as phosphate and nitrogen, biochar may stop these elements from leaking into groundwater. In addition to helping to manage agricultural runoff responsibly, this lowers the chance of nutrient contamination.

Adjusting Soil pH: Biochar is a useful tool for adjusting soil pHIt can raise the pH of acidic soils, making them more suitable for a wider range of crops and unable to require lime treatments.

Carbon Sequestration: One of biochar's main advantages is its long-term capacity to store carbon in the soil for millennia. Parthenium hysterophorus biochar may be added to the soil to store carbon and help mitigate the effects of climate change.

Enhanced Microbial Activity: Biochar has the ability to foster the growth of advantageous soil microbes. By enhancing soil health and nutrient cycling, this may eventually aid in the development of plants.

Water Management: By enhancing soil water retention, biochar lowers the need for regular watering. This is particularly helpful in areas where rainfall is erratic or scarce.

Decreased Soil Erosion: Biochar may lessen the possibility of soil erosion by improving the soil's structure. This is necessary to maintain soil quality and prevent topsoil loss.

Livestock Bedding: Biochar derived from Parthenium hysterophorus may be used as bedding for animals. It has advantages including better control over moisture and less smell.

Compost Amendment: To raise the calibre of the final compost, biochar may be added to compost heaps. It contributes to microbial activity and nutrient retention, making the compost product more enriched.

Bioremediation: In bioremediation, biochar is used to lessen the bioavailability of pollutants in soil, hence lowering its toxicological potential for plants and lowering the likelihood of contamination spreading.

Hydroponics and Aquaponics: Biochar may be used as a growth media in soilless growing systems such as hydroponics and aquaponics. In addition to providing a steady foundation for plant roots, it may enhance water and nutrient management.

Addition for animal Feed: Biochar may be used in some circumstances as an addition for animal feed. It could facilitate better digestion and lessen the negative effects of certain toxins on the digestive systems of the animals.

Carbon Credits: Applying biochar to the soil may help trap carbon and earn carbon credits in specific areas. This may provide an extra financial incentive to utilize it.

Conclusions and Future Perspectives

Invasive weeds and acidic soil have a significant effect on crop productivity and sustainability. Soil acidity must be reduced in order to increase crop production and fertility; this is achieved by keeping noxious weeds from producing valuable biochar. Based on the current review, *P. hysterophorus* biochar considerably increased the SMC, pH, Av. P, exchangeable bases, and SOC when compared to the control treatments that did not include biochar. Nonetheless, there was little fluctuation in the proportions of soil particle sizes throughout time. The bioavailability of metal pollutants, which lead to acidic soil, was considerably reduced by *P. hysterophorus* biochar. Additionally, wheat cultivars treated with biochar on acidic soils showed improved agronomic results. To lessen soil acidity, integrated agricultural inputs are required, such as P. hysterophorus biochar. To find out how well *P. hysterophorus* biochar improves acidic soil, soil microbes, and yields in various soil types under field circumstances, further research will be required. It is important to consider the specific soil, environmental, crop, and plant demands while applying Parthenium weed biochar, or any other kind of biochar, in order to determine the ideal application rate and procedure for maximum benefits. To ensure that biochar is effective in a variety of applications, quality monitoring is also necessary throughout the production process.

REFERENCES

Ahmad, M., Rajapaksha, A. U., Lim, J. E., Zhang, M., Bolan, N., Mohan, D. & Ok, Y. S. (2014). Biochar as a sorbent for contaminant management in soil and water: a review. Chemosphere, 99, 19-33.

Ajayi A. E. and Horn R. (2016). Modification of chemical and hydrophysical properties of two texturally differentiated soils due to varying magnitudes of added biochar. Soil and Tillage Res. 164: pp 34-44.

Ameta, K. S., R. Ameta and S. Ameta. (2016). Effect of compost of *P. hysterophorus* on growth and yield of wheat (*Triticum aestivum*): A comparative study. Journal of American Science.

Anonymous. (2020). www.pakissan.com, allabout/crops/maize Pakistan Agriculture Research Council, Islamabad. Accessed on September 15, 2020.

Atkinson C. J, Fitzgerald J. D. and Hipps N. A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: A review. Plant and Soil. 337(1): pp 1-18.

Bajwa, A. A., Chauhan, B. S., Farooq, M., Shabbir, A., & Adkins, S. W. (2016). What do we really know about alien plant invasion? A review of the invasion mechanism of one of the world's worst weeds. Planta, 244, 39-57.

Bajwa, A. A., Chauhan, B. S. and Adkins, S. (2017). Morphological, physiological and biochemical responses of two Australian biotypes of *P. hysterophorus* to different soil moisture regimes. Environmental Science and Pollution Research 24, 16186-94.

Batish, D. R., Singh, H. P., Pandher, J. K., and Kohli, R. K. 2005a. Allelopathic Interference of *P. hysterophorus* residues in soil. Allelo. J. 15: 267-273.

Chan, K.Y., Xu, Z., (2009). Biochar: nutrient properties and their enhancement. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, Division, Islamabad

Division, Islamabad

Downie, A., Crosky, A., Munroe, P. (2009). Physical properties of biochar. In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, London, pp. 13e32.

Dume B, Ayele D, Regassa A. and Barecha G. (2016). Interactive effects of biochar in soil related to feedstock and pyrolysis temperature. American-Eurasian J. Agric. Environ. Sci. 16 (3): pp 442-448.

FAO. (2008). A guide to laboratory establishment for plant nutrient analysis FAO fertilizer and plant nutrition bulletin no 19.

Glaser B, Lehmann J. and Zech W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – a review. Biology and Fertility Soils. 35: 219-230.

Jeffery, S., Verheijen, F. G., van der Velde, M., & Bastos, A. C. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, ecosystems & environment*, *144*(1), 175-187.

Kasozi, G.N., Zimmerman, A.R., Nkedi-Kizza, P., Gao, B., (2010). Catechol and humic acid sorption onto a range of laboratory-produced black carbons (biochars). Environmental Science and Technology 44, 6189e6195.

Khan, H., K. B. Marwat, G. Hassan, M. A. Khan and S. Hashim. (2014). Distribution of *Parthenium* weed in Peshawar valley, KPK-Pakistan. Pak. J. Bot., 46(1): 81-91.

Khan, H., Marwat, K. B., Khan, M. A., & Hashim, S. (2014). Herbicidal control of *Parthenium* weed in maize. Pak. J. Bot, 46(2), 497-504.

Khan. H., K. B. Marwat, G. Hassan and M. A. Khan. (2013). Socio-economic impacts of parthenium weed (*Parthenium hysterophorus* L.) in Peshawar valley, Khyber Pakhtunkhwa. Pak. J. Weed Sci. Res., 19(3): 275-293.

Khan. H., K. B. Marwat, G. Hassan and M. A. Khan. 2012. Chemical control of Parthenium hysterophorus L., at different growth stages in non-cropped area. Pak. J. of Bot., 44(5): 1721-1726.

Kohli, R.K., Batish, D.R., Singh, H.P., and Dogra, K. 2006. Status, invasiveness and environmental threats of three tropical American invasive Weeds (*P. hysterophorus* L., *Ageratum conyzoides* L., *Lantana camara* L.). Biol. Invasions. 8: 1501-1510.

Kumar S, Masto RE, Ram L. C. Sarkar P, George J and V. A. Selvi. (2013). Biochar preparation from *Partheniumhysterophorus* and its potential use in soil application," *Ecological Engineering*, vol.55, pp. 67–72.

Laghari M, Hu Z, Mirjat M S, Xiao B, Tagar A A and Hu M (2016). Fast pyrolysis biochar from sawdust improves the quality of desert soils and enhances plant growth. J. Sci. Food Agric. 96(1): pp 199-206.

Lehmann J Da Silva Jr JP, Steiner C, Nehls T, Zech W and Glaser B. (2003). Nutrient availability and Leaching in an Archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, Manure and Charcoal Amendments. *Plant and Soil*, 249: 343-357.

Lehmann J, Gaunt J. and Rondon M. (2006). Biochar sequestration in terrestrial ecosystems: A review. Mitig. Adapt. Strategies Global Change. 11(2): pp 395-419.

Lehmann J, Rillig M C, Thies J, Masiello C A, Hockaday W C and Crowley D (2011). Biochar effects on soil biota - A review. Soil Biol. Biochem. 43: pp 1812-1836.

Lehmann, J. and Joseph, S. (2009). Biochar for Environmental Management: Science and Technology. ES BEM 13-1 13/1/09.

Lehmann, J., 2007a. Bio-energy in the black. Frontiers in Ecology and the Environment 5, 381e387.

Major, J., Lehmann, J., Rondon, M., Goodale, C., (2010). Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Global Change Biology 16, 1366e1379.

Marwat, K. B., Hashim, S., & A. Haidar. (2010). Weed management: a case study from north-west Pakistan. Pak. J. Bot, 42, 341-353.

Marwat, K.B.(1984). Studies on the weeds of important cereal crops of NWFP. Ph.D. Thesis, Department of Botany, Univ. of Peshawar, Pakistan.

Marwat, K. B., G. R. Marwat and F. M. Sarim. (1993). A checklist and key to parasitic weeds of Pakistan. Pak. J. Weed Sci. Res. 6 (1-2):1-15.

McCauley, A., Jones, C., & Jacobsen, J. (2009). Soil pH and organic matter. Nutrient management module, 8(2), 1-12.

Navie, S. C., McFadyen, R. E., Panetta, F. D. and Adkins, S. W. (1996). The Biology of Australian weeds 27. *P. hysterophorus* L. Plant Prot. Quart. 11: 76-88.

Oudhia, P. 2001. Phytosociological survey of rainy season weeds of wastelands with special reference to Pathenium hysterophorus in Raipur district. http:// www.IPRNG.org.

Patel S. (2011). Harmful and beneficial aspects of *P. hysterophorus*: an update. *Biotech*, vol. 1, no. 1, pp. 1-9.

Rai, P. K., & Singh, J. S. (2020). Invasive alien plant species: Their impact on environment, ecosystem services and human health. Ecological indicators, 111, 106020.

Ramaswami, P. P. 1997. Potential uses of *Parthenium*. In: Proc. First Int. Conf. on *Parthenium* Management. pp. 77-80.

Rao, V.S. (2000). Weed Parasitism. In Principles of Weed Science 2nd ed. Oxford and IBH publishing Co.Pvt.Ltd. New Delhi & Calcuta. pp.17.

Sameera P. M., A. Suresh, J. Chapla and P. Raja Rao, (2021) Review on Biochar Production and its Applications in Agriculture. Int. J. Curr. Microbiol. App. Sci 10(10): 326-338.

Seebens, H., Bacher, S., Blackburn, T. M., Capinha, C., Dawson, W., Dullinger, S., and Essl, F. (2021). Projecting the continental accumulation of alien species through to 2050. Global Change Biology, 27(5), 970-982.

Shabbir, A., & Bajwa, R. (2007). Parthenium invasion in Pakistan-a threat still unrecognized. Pak J Bot, 39(7), 2519-2526.

Sohi S, Krull E, Capel E L and Bol R (2010). A review of biochar and its use and function in soil. Adv. Agron. 105(1): pp 47-82.

Tamado T, Schutz W, Milberg P. (2002). Germination ecology of the weed *P. hysterophorus* in Eastern Ethiopia. Annals of Applied Biology 140(3): 263 -270.

Tefera, T. (2002). Allelopathic effects of *P. hysterophorus* extracts on seed germination and seedling growth of Eragrostis tef. J. Agron. Crop Sci. 188: 306-310.

Thies J and Rillig M C (2009). Characteristics of biochar: biological properties. In: Biochar for Environmental Management: Science and Technology (Lehmann J, Joseph S. eds.). Earthscan Ltd, London: pp 85-105.

Tobacco: 38-39pp.

Tong H, Hu M, Li F, Liu C and Chen M (2014). Biochar enhances the microbial and chemical transformation of pentachlorophenol in paddy soil. Soil Biol. Biochem. 70: pp 142-150.

Venkatesh G. Sammi Reddy K, Gopinath K. A, Visha Kumari V and Chary R. G. (2018). Biochar for enhancing the soil, water and crop productivity in rainfed areas. In: Krishna Rao B, Sammi Reddy K, Visha Kumari V, Nagarjuna kumar R and Rejani R. (eds.). Rainwater Management for Climate Resilient Agriculture in Drylands. ISBN: 978-93-8088.

Yam, T.Y., Ernst, R., Arditti, J., Nair, H., Weatherhead, M.A., 1990. Charcoal in orchid seed germination and tissue culture media: a review. Lindleyana 5, 256e265.

Zhang M, Cheng G, Feng H, Sun B, Zhao Y, Chen H, Jing Chen J, Dyck M, Wang X, Zhang J and Zhang A (2017). Effects of straw and biochar amendments on aggregate stability, soil organic carbon, and enzyme activities in the Loess Plateau, China. Environ. Sci. Pollut. Res. DOI 10.1007/s11356-017-8505-8.

Zwieten V. L, Kimber S, Morris S, Chan K. Y, Downie A, Rust J, Joseph S and Cowie A. (2010). Effects of biochar from slow pyrolysis of paper mill waste on agronomic performance and soil fertility. Plant and Soil. 327: 235-46.

CHAPTER 5

TÜRKİYE's NATIVE PLANT SPECIES *Dictamnus albus*[1]

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INTRODUCTION

Dictamnus species have important medicinal value with biohemical compounds and ornamental plant as well as cut flower potential with beautiful flowers and long stems.

D. *albus* is one of important native plant species in very rich biodiversity of Türkiye. It has a widespread in Central Europe, North America and Asia. It is naturally grown in the provinces of Karabük, Amasya, Hatay, Isparta, Samsun and Trabzon in Turkey. It is a half woody herbaceous perennial plant with nice flowers.

Dictamnus albus (gas plant) is mostly distributed in open forested areas. In recent years, due to the activities such as grazing and foresting, it has been in danger of extinction. In the last 60 years, about 15-20% of the population has been lost. Small population size puts the future existence of the plant at risk. Dictamnus (Rutaceae) has only a species in Turkey which is *Dictamnus albus*. It is locally called in Turkey as "Akgiritotu or Gazelotu”. It is known as flowering branches and used for some stomach disorders, as a tonic and antipyretic. White, pink or rose-colored flowers bloom in spring on an upright bunch of flower stem. *Dictamnus albus*, commonly called as bugas plant, produces a flammable volatile gas from flowers and seed capsules.

Although *Dictamnus albus* is an attractive garden plant, its use in trade is limited because of the difficulties in propagating by classical methods. This species has the potential to be used as an outdoor plant for landscaping as well as using as cut flowers with long stems in vases (Sarı and Çelikel, 2018).

In this review, some information on distribution, ornamental properties, medicinal values and other aspects of *Dictamnus* species and *D. albus* are given to emphasis the importance of the species.

1. *Dictamnus albus* in BOTANY

Dictamnus albus ('Burning Bush', Rutaceae) is an erect, branched, 30-90 cm tall, long-lived and perennial plant characterized by a pseudorhizome and thick fleshy storage roots. Its distribution areas are in woodlands, open oak forests and pastures in warm temperate regions.

Plants may have white, pink or rose-colored flowers arranged in a pyramid shape on one or several flower stalks. Flowering continues from early

May to early July. It has low seed production (about 50 per flower). The potential lifespan of the plant is at least 30 years (Jaer et al., 1997).

Figure 1: Burning Bush (*Dictamnus albus)* Plants and Flowers Grown in Slovenia. Photographer: Dr. Amadej Trnkoczy. ID: 0000 0000 0207 0141 (2007-02-05). https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+0207+0139 (top) https://calphotos.berkeley.edu/cgi/img_query?enlarge=0000+0000+0207+0141 (bottom)

Dictamnus albus is predominantly foreign pollinated. Pollination occurs by insects. Its reproduction in nature occurs through the dispersal of its seeds (Frey, 2000). It produces a volatile gas that can burn from flower and seed capsules (Figures 1 and 4).

2. *Dictamnus* DISTRIBUTION in the WORLD

Dictamnus species in the world has been distributed in middle Europe, northern America and Asia. Burning Bush (*Dictamnus albus*) plants and flowers grown in Slovenia are shown in Figure 1. There are 28 plant species belong to the genus *Dictamnus* (Hassler, 2018).

Dictamnus species are native to: Albania, Altay, Austria, Bulgaria, Czechoslovakia, East European Russia, France, Germany, Greece, Hungary, Iran, Italy, Kazakhstan, Kirgizstan, Krym, Lebanon-Syria, North Caucasus, Pakistan, Palestine, Poland, Romania, Sinai, South European Russi, Spain, Switzerland, Tadzhikistan, Transcaucasus, Türkiye, Ukraine, Uzbekistan, West Himalaya, Yugoslavia. Dictamus species are introduced into: Michigan, New York, Vermont (Figure 2; POWO, 2023).

3. *Dictamnus albus in TÜRKİYE*

The distribution of gazelle grass (*Dictamnus albus*) in Türkiye (TÜBİVES, 2023) is shown in Figure 3. As can be seen, the Gazelle plant has a natural distribution in the north and south of the country (Figure 3). *Dictamnus albus* flowers and plants naturally grown in Trabzon Ayder Plateau (photo taken by İ.GEDİKOĞLU) are given in Figure 4.

Türkiye is a very rich country in floristic terms with 12.000 plant species and 3.925 (34%) endemic plants. Within this richness, *Dictamnus albus* (*Rutaceae*) is represented as the only species. According to TUBIVES records, this species is naturally distributed in the provinces of Karabük, Amasya, Hatay, Isparta, Samsun and Trabzon (Davis, 1982). In Turkey, the plant is locally known as "Akgiritotu or Gazelotu" (Baytop, 1984).

Figure 2: Flower Structure (above) and Distribution (bottom) of the *Dictamnus albus* L. Over the World (POWO, 2023). Native (green) Introduced (purple)
Source: https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:772356-1

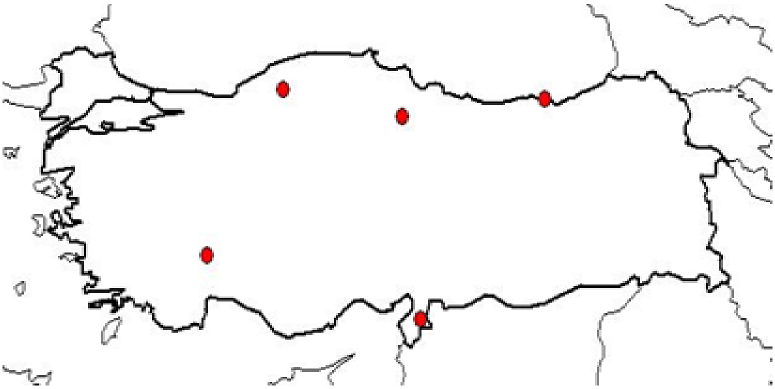

Figure 3: Distribution of the *Dictamnus albus* Over Türkiye According to the Turkish Plants Data Service 'TÜBİVES'.

According to some studies conducted in Türkiye, some distribution areas of *Dictamnus albus* (Gazelle grass) determined are around:

- ✧ Armutlu çayır, located within the borders of Kastamonu province at 1277 m (Özen et al., 2013),
- ✧ Zonguldak province Devrek-Mengen road, near the Eğerci turnout, at 184 m (Sarıbaş and Kaplan, 2008),
- ✧ under the oak and dogwood forest of Ovacık Keşmen field in Karabük province (Dikilitaş et al., 2016),
- ✧ Kırklareli-Dereköy at 469 m (Uruşak et al., 2013),
- ✧ Karabük, Sırçalı Canyon, among *Pinus nigra*, at 610 m (Filiz, 2007),
- ✧ Sinop, Durağan, Kepez valley *P. brutia* forest, at 300 m (Korkmaz and Engin, 2001),
- ✧ Trabzon Maçka district, Altındere Valley, 'Coşan dere' village, deciduous forests, at 500-630 m (Uzun and Terzioğlu, 2008).

4. PROPAGATION METHODS of *Dictamnus albus*

Although the gazelle plant has the feature of being a showy garden plant, its commercial availability has been limited because it is difficult to produce using classical methods. Gas plant can be propagated by seed, division and rhizome. However, these methods are insufficient for commercial breeding purposes. The fact that propagation by seeds takes a long time and a small number of plants can be produced by separation and division of rhizomes makes it difficult to obtain sufficient plants for commercial cultivation (Huxley, 1992).

Figure 4: *Dictamnus albus* Plants Naturally Grown in Trabzon Ayder Plateau. Photographer: İ.Gedikoğlu. https://yabanicicekler.com/flower/dictamnus-albus-396

In propagating *Dictamnus albus* from seed, a 6-week cold application positively affects germination. Seeds germinate in 1-6 months at 15°C after cold application. Germinated seedlings are transplanted into viols or pots and planted in their permanent locations in the spring. The plant can also be propagated by dividing the rhizomes between November and December. New plants formed in the spring are grown by transplanting them into pots or seedling pans (Huxley, 1992).

5. CULTIVATION and ORNAMENTAL USAGES

Dictamnus albus is used for landscaping purposes in parks and gardens with its showy flowers. It has also potential plant that can be used as potted plants indoor and cut flowers in vase with its long stems and beautiful flowers, as long as it has a satisfactory vase life which should be studied.

Some cultivars have been developed for growing in parks and gardens. The variety *D. albus* var. Purpureus has gained the Royal Horticultural Society's Award of Garden Merit. Because of the difficulty in propagation and toxicity of the foliage, *Dictamnus* is rarely seen in American gardens (Wikipedia, 2023).

6. CHEMISTRY and MEDICINAL USAGES

In addition to rheumatic diseases, it can also be used as a mestrual stimulant, diuretic and expectorant. *Dictamnus albus* contains essential oil and this oil is used in cosmetics. Among the people, it was used in folk medicine in the treatment of jaundice and skin diseases, and its flowering branches were used in folk medicine as a tonic, stimulant and antipyretic for stomach diseases (Baytop, 1987).

Dictamnus species have more than 100 chemical compounds including alkaloids, limonoid triterpenoids, flavonoids, sesquiterpenoids, coumarins, and phenylpropanoids (Gao et al., 2011).

Cortex Dictamni has been used to treat eczema, rubella, scabies, acute rheumatoid arthritis, skin inflammation, jaundice, cold, and headache in traditional Chinese medines. Quinoline alkaloids and limonoids are the main bioactive components of *Dictamnus* species. These two compounds have important potential and interest to develop new medicines (Lv et al., 2015; Figure 5).

The toxic effect of foliages in *Dictamnus* species needs to be further studied (Lv et al., 2015). Despite the lemon-like smell of *Dictamnus* plants, the leaves have a bitter and unpleasant taste. Contact with the leaves may cause phytophotodermatitis. The *Dictamnus albus* plant is called burning bush due to the contents of volatile oils isoprene produced by the plant, which can catch fire in hot weather (Wikipedia, 2023).

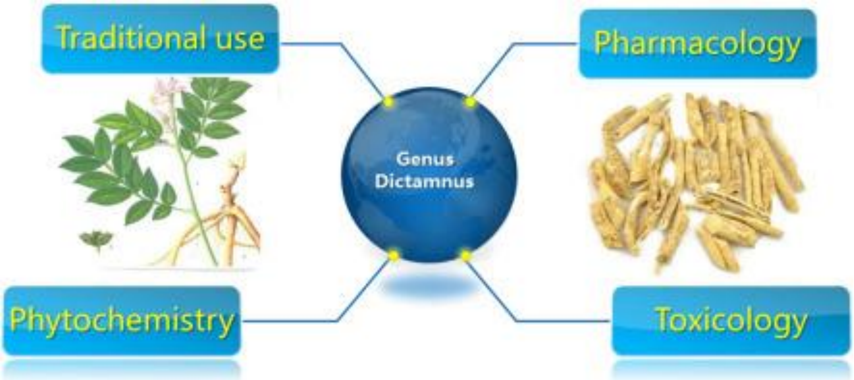

Figure 5: Medicinal uses, phytochemistry and pharmacology of the genus *Dictamnus* (Rutaceae) (Lv et al., 2015).

7. SUGGESTIONS and CONCLUSION

Dictamnus albus has a high potential for use as an ornamental plant, especially in landscaping, with its showy flowers in the form of clusters on an upright stem. Especially in developed countries, the use of completely natural plants that are low in cost and adaptable to the region in which they are used is becoming widespread.

Due to the long duration and high cost of breeding outdoor ornamental plants, the propagation of natural plants and their use in landscaping areas, as potted flowers and even as cut flowers has become increasingly common in recent years. The flora of Türkiye is quite rich compared to Europe and surrounding countries. However, natural plants are not utilized sufficiently. It is a fact that many ornamental plants imported from abroad which is one of the important obstacles to the development of the ornamental sector. Sustainable usage of native plants is of importance as well as protecting valuable genetic resources (Çelikel, 2015; 2023).

It is clear that the use of our existing species richness in the ornamental plants sector, especially in outdoor plants, will make a positive contribution to the sector. With its features, *Dictamnus albus* is a plant that can be used both outdoors and as a cut flower in the ornamental plants sector. However, there is no research in our country regarding the evaluation of the plant in the ornamental plant sector.

In Türkiye, *Dictamnus albus* and many other flowering plants are endangered due to reasons such as intense construction in their natural distribution areas, human activities, tourism activities, grazing and collecting. For this reason, apart from studies aimed at protecting the generations of these plants, many of which are naturally distributed in our country, are endemic and endangered, research studies on cultivation and propagation should be accelerated. Besides Burning Bush plants and many other native plants could be used as medicinal plants and ornamental plants as cut flowers or in door and outdoor plants. In addition, as a valuable genetic resources they should be saved in the flora and their collection and usages should be sustainable considering the future.

REFERENCES

Baytop, T. (1989). Therapy with Plants in Turkey, *Istanbul University Publication*, Istanbul, p.418.

Çelikel, F.G. (2015). Süs bitkilerinde tohumluk (tohum, fide, fidan, soğan) üretimi ve kullanımı. *SÜSBİR Dergisi*, 3: 32-33.

Çelikel, F.G. (2023). Lecture notes on ornamental plants. Ondokuz Mayıs University, Department of Horticulture, Samsun.

Davis, P.H. (1982). *Buxus* L. in: Davis PH (ed.) Flora of Turkey and the East Aegean Islands, *Edinburgh University Press Edinburgh*, Vol. 7, pp. 631-632.

Dikilitaş, B., Güler, B., Uğurlu, E., Altan, Y. (2016). Ovacık (Karabük) ve Çevresinin Floristik Özellikleri. Celal Bayar Üniversitesi Fen Bilimleri Dergisi, 12(2): 253-264.

Filiz, Z. (1997). Sırçalı Kanyonu Florası (Safranbolu). Yüksek Lisans Tezi, Fen Bilimleri Enstitüsü, Gazi Üniversitesi, Ankara, 96s.

Frey, W. (2000). Life Strategies as a Basis for Functional Characterization of Plant Species and Plant Communities: a Case Study. *Zeitschrift für Ökologie und Naturschutz*, *9*(1/2): 35-41.

Gao X.; Zhao P.-H.; Hu J.-F. (2011). "Chemical constituents of plants from the genus *Dictamnus*". Chemistry and Biodiversity. 8 (7): 1234–1244. doi:10.1002/cbdv.201000132.

Hassler, M. (2018). World Plants: Synonymic Checklists of the Vascular Plants of the World (version Jan 2015). In: Species 2000 & ITIS Catalogue of Life, 2015 Annual Checklist (Roskov Y., Abucay L., Orrell T., Nicolson D., Kunze T., Flann C., Bailly N., Kirk P., Bourgoin T., DeWalt R.E., Decock W., De Wever A., eds). Digital resource at www.catalogueoflife.org/col. Species 2000: *Naturalis, Leiden, The Netherlands.*

Huxley, A. (1992). Excellent and very comprehensive, though it contains a number of silly mistakes. Readable yet also very detailed. *The New RHS Dictionary of Gardening*, MacMillan Press.

E.J. Jäger A., Lorenz, J. H. (1997) Wuchsform und Lebensgeschichte von *Dictamnus albus* L *(Rutaceae). Hercynia* 30: 217–226

Korkmaz, H., Engin, A. (2001). The Flora of the Boyabat (Sinop) Dam and its Environs. *Turkish Journal of Botany,* 25(6): 397-435.

Lv, M., Xu, P., Tian, Y., Liang, J., Gao, Y., Xu, F., Zhang, Z., Sun, J. (2015). Medicinal uses, phytochemistry and pharmacology of the genus *Dictamnus* (Rutaceae), Journal of Ethnopharmacology, 171: 247-263, ISSN 0378-8741, https://doi.org/10.1016/j.jep.2015.05.053.

Özen, M. D., Özbek, M. U., Vural, M. (2013). Flora of Armutlu çayır (Kastamonu/Turkey). *Biological diversity and conservation*, *6*(1): 22-31.

POWO (2023). Plants of the World Online. Facilitated by the Royal Botanic Gardens, Kew. Published on the Internet; http://www.plantsoftheworldonline.org/ Retrieved 05 November 2023. https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:772356-1#publications.

Sarı Ö., Çelikel, F.G. (2018). Türkiye'de doğal yayılış gösteren süs bitkisi gazel otu (*Dictamnus albus*). The 2nd International UNIDOKAP Black Sea Symposium on BIODIVERSITY 28-30 November 2018 - Ondokuz Mayıs University, Samsun, Türkiye.

Sarıbaş, M., Kaplan, A. (2008). Zonguldak ili florasına katkılar. *Biyolojik Çeşitlilik ve Koruma* 1:40-65.

TUBİVES, (2023).Türkiye Plants Data Service-Taxa in Provinces. http://www.tubives.com

Uruşak, E. A., Özhatay, F. N., Güler, N., Ersoy H., Başak N., Yeşil Y., Demirci, S. (2013). The flora of Yıldız Mountains (Kırklareli) Biosphere Project Area. *Turkish Journal of Botany*, 37(2):225-269.

Uzun, A., Terzioğlu, S. (2008). Vascular Flora of Forest Vegetation in Altındere Valley (Maçka-Trabzon). *Turkish Journal of Botany, 32*(2): 135-153.

Wikipedia, 2023. https://en.wikipedia.org/wiki/*Dictamnus_albus*

CHAPTER 6

INSIGHTS INTO IMMOBILIZATION TECHNIQUES AND INDUSTRIAL APPLICATIONS OF *Rhizopus oryzae*

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INTRODUCTION

Immobilization plays a pivotal role in the realm of biotechnology, particularly when it comes to enzymes or cells. This chapter discusses the significance of immobilization in different industrial processes, focusing on its application in the synthesis of physiologically active compounds such as organic acids, extracellular enzymes, etc. The use of immobilized microorganisms, particularly in industrial fermentation, has proven to be a breakthrough, enabling continuous processes with significant economic benefits. The examples of the efficacy of immobilized microorganisms in the industrial production of substances like L-aspartic acid, L-malic acid, and semisynthetic penicillin demonstrate the practical significance of this biotechnological technique.

This chapter also provides a glimpse into the diverse applications of *R. oryzae*, with a specific focus on its role in L(+)-lactic acid production and the various immobilization strategies employed to enhance fermentation processes. In the sections that follow, it will be discussed that individual research projects and conclusions about *R. oryzae* immobilization, provide insight into the complexities of these procedures and how they may affect the development of biotechnology.

The examination of immobilization techniques and their uses lays the basis for an in-depth understanding of their function in process optimization and innovation in biotechnological advances.

1. Immobilization

The limitation or prevention of the movement is called immobilization. In the field of biotechnology, when enzymes or cells are immobilized, it indicates that they are affixed to a substrate or one another (Virkajärvi & Linko, 1999). The use of immobilized cells in industrial processes dates back to ancient times. For instance, there is documentation of the immobilization of bacteria for vinegar production dating back to as early as 1670 (Mitchell, 1926).

1.1. The importance of immobilization

The significance of immobilization in biotechnological processes lies in its potential to optimize these processes, leading to increased productivity, higher product concentrations, and improved conversion of raw materials

(Katzbauer et al., 1995). The objective of immobilizing living cells is to create a biocompatible environment that preserves biocatalytic activity while facilitating the flow of essential nutrients. In several instances, encapsulated cells sustain viability without proliferation, ensuring prolonged catalytic activity without a loss of viability (Sizemore et al., 2013). The use of immobilized microorganisms, particularly for producing biologically active substances like extracellular enzymes, has gained attention due to its applicability across various industrial processes (Lusta et al., 2000). Immobilization facilitates continuous processes, offering substantial economic benefits, and has proven to be a breakthrough in some cases where continuous processes with free cells were initially disappointing (Virkajärvi & Linko, 1999). Moreover, the practice of immobilizing microorganisms has typically been appealing in industrial fermentation as a means to enhance the production yield of desired products while allowing for the repeated utilization of the immobilized cells (Tanyıldızı et al., 2012; Trakarnpaiboon et al., 2018).

The success of immobilized microbial cells have been demonstrated in the industrial production of various substances, such as L-aspartic acid, L-malic acid, L-alanine, D-aspartic acid (used in semisynthetic penicillin), high glucose syrup, palatinose, and acrylamide (Tosa & Shibatani, 1995).

Lusta et al. (2000) proposed creating a gel matrix with macroporous structure designed to serve as a suitable support for immobilizing filamentous fungi. The unique features of this gel matrix were focused on regulating porosity and ensuring its capacity to withstand extended periods of repeated batch processing for the production of extracellular cellulases and xylanase (Lusta et al., 2000).

The immobilization of fungal spores in the carrier matrix enables the formation of small granules containing metabolically active mycelium. This arrangement promotes an advantageous distribution of hyphae, facilitating the active synthesis of organic acids (OAs) (Naude & Nicol, 2017; Pimtong et al., 2017). The use of poly(vinyl alcohol) (PVA) cryogel as a carrier for immobilizing filamentous fungal cells has proven to be highly effective (Dotsenko et al., 2018), because this carrier exhibits both high chemical and mechanical stability, along with a well-developed macroporous structure that facilitates mass transfer (Lozinsky, 2008). Another study focuses on the production of various organic acids such as succinic, fumaric, or lactic, by microbial cells such as filamentous fungi *R. oryzae* and bacteria *Actinobacillus succinogenes* immobilized in “PVA cryogel using diverse renewable raw materials (wheat and rice straw, aspen and pine sawdust, Jerusalem artichoke stems and tubers, biomass of macro- and microalgae) in batch conditions”. Compared to free cells, immobilized cells exhibited higher process productivity, bulk output, and concentrations of organic acids under identical conditions (Maslova et al., 2019).

1.2. Methods of cell immobilization

Various methods of cell immobilization are commonly employed in biotechnology (Katzbauer et al., 1995), primarily achieved through entrapment within diverse organic or inorganic matrices or by attaching them to surfaces. Binding methods include physical adsorption or chemical binding, and combinations like adsorption followed by cross-linking (Figure 1). Entrapment techniques, widely utilized for whole-cell immobilization, involve enclosing microorganisms in a porous network through physical or chemical polymerization. This method, known for its simplicity and minimal biocatalyst requirement, starts with suspending microorganisms in a selected matrix solution. When selecting a matrix solution, various properties such as price, cell load, ease and type of immobilization, mass transfer, regeneration, reactor channeling or blocking, stability, sterilization, rigidity, and approval for food use should be considered (Virkajärvi & Linko, 1999). Examples of matrices include cellulose derivatives, diatomaceous earth, porous glass, silicon carbide, silicon rubber, and wood chips. Factors like cost, regeneration necessity, and suitability for food use influence matrix choice. Wood chips, for instance, are a cost-effective matrix for yeast in continuous reactors and may not require regeneration due to their low cost, but regeneration should be simple if needed.

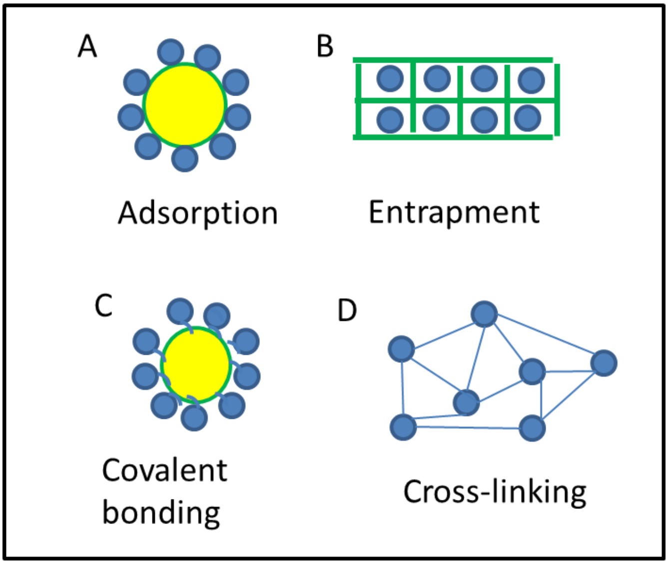

Figure 1. Immobilization methods: (A) Adsorption, (B) Entrapment, (C) Covalent binding, (D) Cross-linking.

Matrices in entrapment methods include synthetic polymers (polyvinyl alcohol, polyacrylamide, polyurethane), thermogels (cellulose, agarose, agar), and hydrogels (chitosan, alginate, κ-carrageenan). Sodium alginate entrapment is a well-known method where cells mixed with water-soluble alginate forms water-insoluble alginate beads in a calcium chloride solution. Agar or agarose entrapment follows a similar procedure in an oil solution. Another common synthetic method employs polyacrylamide gel, involving the polymerization of acrylamide monomer, whole cells, and a cross-linker reagent (Trelles & Rivero, 2020). However, isotropic xerogel carriers, while widely used, have drawbacks such as low durability: decreased microorganism activity due to matrix formation, requiring optimization for each microorganism and mass transfer issues, along with inducing stress and toxicity in immobilized cells (Lusta et al., 2000). Techniques to address mass transfer resistance include immobilizing cells in thin films, attaching them to the surface of membranes, or using a fibrous matrix achieved through natural attachment or crosslinking to fibers. Although there are difficulties in matching the size and form of the cells, macroporous sponge carriers such as polyurethane (Trakarnpaiboon et al., 2018) or cellulose foams (Chiou et al., 1998) have been used to address these problems.

1.3. Advantages of Immobilization

Immobilization is favored for enhancing process efficiency by addressing challenges associated with submerged fermentation, including aggregate formation and microbial growth inhibition due to metabolite accumulation (López et al., 2008). Immobilized whole cells offer advantages compared to ordinary suspension culture systems, such as avoiding cell wash-out at high dilution rates, achieving higher cell concentrations in the reactor, facilitating separation of cells from product-containing solution or the system, the ability to reuse biocatalysts, reduced susceptibility of cells to contamination, improved process control, and high volumetric productivity (Furusaki & Seki, 1992; Yen & Lee, 2010).

2. *Rhizopus oryzae* and its Industrial Uses

The genus *Rhizopus* belongs to the Phylum Zygomycota, class Zygomycete, order Mucorales, family Mucoraceae, and genus *Rhizopus*. *Rhizopus* is characterized by the formation of rhizoids (Figure 2), and while some strains are found in plants or animals, *R. oryzae*, a species within this genus, is remarkable for efficiently producing various bioproducts. It is utilized in the production of traditional food items like tempeh, peka, ragi, and loog-pang, organic acids (lactic acid (LA), fumaric acid, gallic acid), alcoholic beverages, and enzymes such as cellulases, tannases, glucoamylases and polygalacturonases (Abd Razak et al., 2017; Ghosh & Ray, 2011; Nursiwi et al., 2021). Due to its commercial significance, *R. oryzae* is considered a microorganism of great interest.

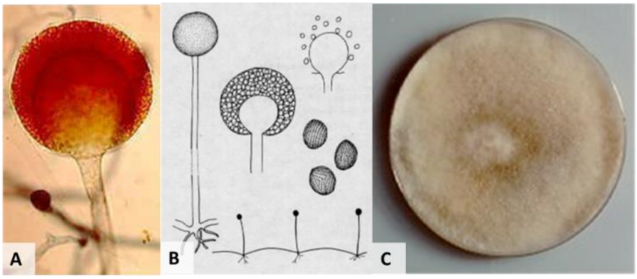

Figure 2. *R. oryzae*, A) The microscopic appearance of sporangia, B) Illustrative scheme of hyphae, columella, and rhizoids C) The colonial appearance

R. oryzae is widely distributed in nature, particularly in tropical and subtropical regions, and is commonly isolated from diverse substrates, including soils, decomposing vegetables, fruits, and seeds. Its rapid growth makes it a primary or secondary colonizer, quickly invading digestible substrates rich in simple sugars (Battaglia et al., 2011). *R. oryzae* is an industrially employed microorganism, because it is recognized as generally safe (GRAS), and is able to utilize a wide range of carbon sources (Ghosh & Ray, 2011).

Moreover, *R. oryzae* is extensively researched for its commercial potential in producing L(+)-lactic acid. In basic media with minimal nutrient supplementation, it is capable of producing optically pure L-(+)-lactic acid. It offers advantages over lactic acid bacteria (LAB) due to lower nutrient requirements, and its product is often optically pure, unlike the racemic mixture produced by LAB. Compared to commonly used bacterial producers, *R. oryzae* can tolerate higher concentrations of accumulated LA. Additionally, *R. oryzae*'s amylolytic ability allows it to produce lactic acid from starchy materials without the need for glucoamylase during fermentation, potentially reducing enzyme costs significantly (Battaglia et al., 2011; Özer Uyar & Uyar, 2023). Currently, there is a growing demand for L-(+)-lactic acid, particularly for the production of the biopolymer polylactic acid (PLA). PLA represents a promising alternative to petrochemical-derived plastics, offering biodegradability, biocompatibility, and environmental friendliness. PLA finds applications in various fields, including disposable consumer products, surgical sutures, drug delivery systems, and orthopedic implants (Balla et al., 2021).

3. Immobilization of *R. oryzae*

LA fermentation by *R. oryzae* has some drawbacks, such as the aerobic nature requiring the cost of aeration (bioreactor agitation and/or air supply). The fungus exhibits different morphologies (filamentous cells, clumps, pulpy structures, flocs, and pellets) influenced by factors like temperature, agitation rate, pH, and medium composition. Filamentous fungi fermentation faces complications like reduced oxygen transfer, wall growth, increased broth viscosity due to mycelial growth, which are critical for LA production. Numerous techniques have been investigated to control cell density and morphology and enhance product yield, production rate, and productivity (Yamane & Tanaka, 2013). Tay and Yang (2002) summarized these methods, including self-immobilization by forming pellets, cell entrapment within polymeric matrices, cell attachment via surface adsorption. Immobilization simplifies bioreactor operation and control, aids in separating culture media from fungal cells, and facilitates the reutilization of fungal cells to enable long-term LA production (Tay & Yang, 2002).

Researchers have explored various immobilization methods for LA production with *R. oryzae*, with entrapment methods, particularly using soft gels like Ca-alginate, being commonly employed in some studies (Ghosh et al., 2015; Hang et al., 1989; López et al., 2008). However, challenges related to oxygen supply limitation in gel-entrapping methods may reduce fermentation rates and L(+)-lactic acid transformation efficiency. Cell immobilization on a support polymer matrix has been proposed to overcome problems which are related to fermentations with filamentous fungi (Ranjit & Srividya, 2016).

Additionally, previous studies with *Rhizopus* species in submerged cultures explored the effects of ion concentration on lactic acid production, the stability of immobilization in repeated batch fermentations, and the possibility of producing valuable chemicals alongside LA.

Various studies and reviews also discussed the literature on organic acid production using immobilized *R. oryzae* and potential immobilization matrices for LA production.

For example, a study investigated L(+)-lactic acid production using *R. oryzae* immobilized on alginate in a tapered-column fluidized-bed batch reactor. Saturation kinetics with substrate and product inhibitions were observed in linear form, and kinetic constants were determined: V_m (maximal rate) was 11.04 g lactic acid/(L-bead·h), K_{in} (substrate inhibition constant) was 20.9 g glucose/L, K_i (product inhibition constant) was 365 g glucose/L, and K_p (product inhibition constant for lactic acid) was 316 g lactic acid/L. Simulation results aligned well with experimental data when considering the initial lag phase in the model. 73 g/L of L(+)-lactic acid was obtained after 45 h, when initial glucose concentration was 150 g/L. Based on the amount of glucose consumed, with a yield of lactic acid was calculated to be 65% (Hamamci & Ryu, 1994).

Following this, Yen and Lee (2010) utilized *R. oryzae* immobilized in calcium alginate for lactic acid fermentation, unhydrolyzed raw sweet potato powders was used as the primary carbon source. The effects of calcium chloride concentration, immobilized bead diameter, and sodium alginate concentration on lactic acid generation were examined in this study. Higher sodium alginate concentrations during gelation resulted in a more rigid immobilized capsule, leading to reduced lactic acid production. Increased calcium chloride concentrations thickened the immobilized capsule, causing higher mass transfer resistance, but effects were not significant below 15%. Larger bead diameters provided more space for cell growth, optimizing LA production, with the maximum observed at a 5-mm diameter. Repeated batch operations demonstrated that immobilized cells exhibited greater stability in LA production compared to free cells. In comparison to free-cell operation, the total cumulative lactic acid generation in seven repeated batches (216 hours) increased by 55% in immobilized-cell operation, with no noticeable amylolytic activity loss. The findings suggest that *R. oryzae* immobilized in Ca-alginate holds promise for efficient LA production from unhydrolyzed raw sweet potato powders (Yen & Lee, 2010).

In order to produce a significant amount of LA from glucose, *R. oryzae* was cultured in both batch and fed-batch modes. In a three-baffled shaking flask at 37°C, the mycelia of *R. oryzae* were trapped *in situ* within sponge-like cubic particles. Fine powdery calcium carbonate ($CaCO_3$) was added either initially or intermittently to control the pH of the culture liquids. High LA accumulations of 145 g/L and 231 g/L were achieved in batch and fed-batch cultures, respectively, with glucose concentrations exceeding 150 g/L. "In these cultures, the yields and productivities of LA were 95.0%, 1.42 g/L.h, and 92.5%, 1.83 g/L.h, respectively" (Yamane & Tanaka, 2013).

Continuing the exploration of alternative matrices, a loofah sponge emerged as a promising candidate for LA production by immobilized *R. oryzae* (Figure 3). The loofah sponge kept its form and texture even after being treated with buffers of various pH and autoclaved for up to four cycles. The optimal conditions for LA production were found to be a medium containing four pieces of loofa sponge per 100 ml, inoculated with 3×10^6 spores ml^{-1}, resulting in a maximum LA production of 81 g L^{-1} in 48 hours of fermentation. Batch fermentation was successfully repeated ten times, achieving remarkably higher productivities (1.7–1.8 g L^{-1} h^{-1}) during the first five batches, with the peak productivity (1.8 g L^{-1} h^{-1}) observed in the third fermentation batch (Ganguly et al., 2007).

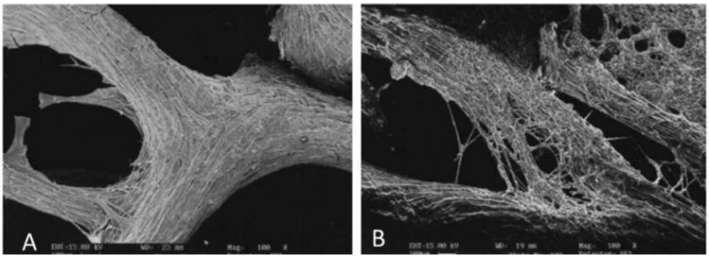

Figure 3. Scanning electron micrograph of R. oryzae immobilized in the loofah sponge; A), Bare loofah sponge; B) Loofah sponge immobilized with R. oryzae (Ganguly et al., 2007).

Expanding on the utilization of loofah sponge, *R. oryzae* demonstrated its proficiency in synthesizing LA from 10 g/l of soluble potato starch through a single-step fermentation process. Immobilizing *R. oryzae* (LIRO) was used to test the natural capacity of fungus to produce biofilms on a loofah sponge (Figure 4). LIRO achieved a maximum LA concentration of 4 g/l within 96 hours, outperforming free cells, which reached 3 g/l. LIRO also exhibited higher specific LA formation and specific starch utilization rate compared to free cells. In a column reactor study using a cell immobilization strategy with optimized temperature and inoculum size, LIRO produced a maximum of 5 g/l of LA within 48 hours in an airlift reactor with a net draft tube (Shahri et al., 2020).

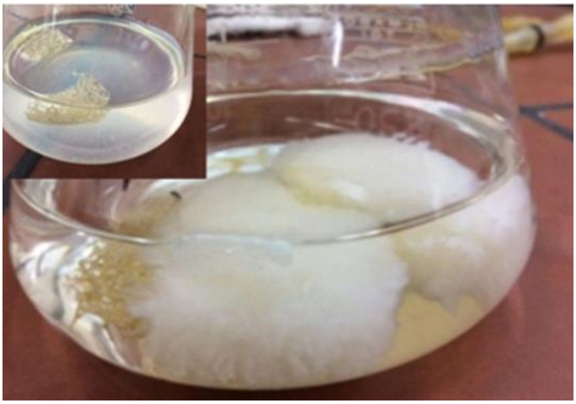

Figure 4. Photograph of the LIRO cells, small photograph shows bare loofah (Shahri et al., 2020).

Wang et al. (2010) developed a novel honeycomb-inspired support matrix for the immobilization of *R. oryzae* cells, aiming to control fungal morphology and improve mass transfer in a bioreactor for lactic acid production. The matrix, featuring asterisk-shaped fibrous structures in a honeycomb configuration, offered a large surface area for cell attachment and biofilm growth. Remarkably, over 90% of inoculated spores were adsorbed onto the matrices within 6–8 hours, achieving 100% immobilization efficiency after 10 hours. In shake-flask experiments with 80 g/L initial glucose, lactic acid production increased by 70% (49.5 g/L vs. 29.3 g/L), and fermentation time decreased by 33% (48 hours vs. 72 hours) compared to free-cell fermentation. The immobilized-cell fermentation was further assessed in a bubble-column bioreactor operated in a repeated batch mode for nine cycles over 36 days. The highest lactic acid production reached 68.8 g/L, with a volumetric productivity of 0.72 g/L h and a lactic acid yield of 93.4% (w/w) from consumed glucose. The overall yield and productivity were 77.6% and 0.57 g/L h, respectively, suggesting the potential for further improvement by optimizing aeration and mixing in the bubble-column bioreactor (Wang et al., 2010).

As another alternative matrix, the researchers focused on the fermentation behavior of *R. oryzae* immobilized in polyurethane foam cubes of various sizes. Through the natural attachment method, *R. oryzae* could be easily immobilized on polyurethane foam cubes larger than 2.5 x 5 x 5 mm^3. The use of small cubes for immobilization proved highly effective in enhancing the productivity of the immobilized cells. However, challenges were encountered in achieving complete immobilization of *R. oryzae* in small cubes, even though increasing the inoculum size improved the immobilization ratio. Microscopic observations revealed that the pore size of the cube matrices was in the range of 0.2 to 0.5 mm. The large pore size did not effectively restrict mycelial growth within the matrices of very small cubes. The authors suggest that a polyurethane foam with smaller pores would contribute to complete immobilization in very small cubes, and efforts are underway to find such a foam to validate this hypothesis (Sun et al., 1996).

Lactic acid production by *R. oryzae* immobilized in polyurethane foam was investigated using response surface methodology with a 23 full-factorial central composite design involving three independent variables: glucose concentration, pH, and agitation rate. The model F-value of 17.01 indicates a good fit. The linear and quadratic effects of glucose concentration and the quadratic effect of agitation rate were found to be significant for lactic acid production. The maximum lactic acid production of 93.2 g/L was achieved with a glucose concentration of 150 g/L, pH 6.39, and agitation rate of 147 rpm. Glucose concentration and agitation rate were identified as limiting parameters, showing that small variations in these parameters could significantly impact lactic acid production. The initial pH did not affect on lactic acid production due to the presence of a neutralizing agent. Lactic acid production from immobilized whole cells under optimal conditions was approximately 55%, higher than that from suspension culture systems (Tanyıldızı et al., 2012).

Ranjit and Srividya (2016) focused on producing L-lactic acid (LA) from agro-industrial waste using both free and polyurethane-immobilized *R. oryzae*, utilizing the natural hydrolytic capabilities of the fungus. The research compares fungal biomass and lactic acid production from various agro wastes, subjected to three different pre-treatments, with the results obtained from starch. Acid-hydrolyzed sugar bagasse demonstrated the highest lactic acid yield at 79.2 g/L, with a peak volumetric productivity of 1.1 g/L/h. Cultivating polyurethane sponge (PUS)-immobilized *R. oryzae* on starch showed a 1.15-fold increase in LA concentration compared to free cells under specific conditions (30°C, 160 rpm agitation rate, pH 6.0 for 72 h). Successful lactic acid production from starch was achieved through four repeated batch cultures with stable efficiency. Across all tested agro waste substrates, PU-immobilized *R. oryzae* exhibited higher lactic acid yields compared to free cells, with sugar bagasse showing a maximum yield of 88.2 g/L (1.22 times higher than with free cells) under specified conditions (30°C, 160 rpm agitation, pH 6.0 for 72 h).

Trakarnpaiboon et al. (2018) produced L-(+)-lactic acid through the fermentation of starchy material, specifically α-amylase-treated liquefied cassava starch and highlighted for cost reduction and increased value of agricultural products. *R. oryzae* was chosen for its ability to produce the highest amount of L-(+)-lactic acid under these conditions. Shaking flask experiments revealed the significance of KH_2PO_4 and $ZnSO_4.7H_2O$, with 150 g/L of α-amylase-treated liquefied cassava starch maximizing L-(+)-lactic acid production. Immobilizing *R. oryzae* on a $0.25 \times 0.25 \times 0.25$ cm^3 PUS in a 3 L airlift fermenter resulted in L-(+)-lactic acid production of 83.7 g/L with a productivity of 1.16 g/L/h after 72 hours. Repeated batch culture with PUS-immobilized *R. oryzae* succeeded through 4 cycles, maintaining high L-(+)-

lactic acid concentration and product yield at 77.7 g/L and 0.62, respectively. Additionally, the study demonstrated the feasibility of achieving L-(+)-lactic acid production in a single stage from liquefied cassava starch without a saccharification process, using *R. oryzae* with glucoamylase activity. Under similar conditions, immobilizing *R. oryzae* on 0.25 × 0.25 × 0.25 cm^3 PUS (Figure 5) led to a high L-(+)-lactic acid production of 83.7 g/L with a productivity of 1.16 g/L/h. The successful repeated-batch culture with PUS-immobilized *R. oryzae* after four cycles, particularly when using α-amylase-treated liquefied cassava starch as a substrate, suggested potential cost reduction in enzyme usage for saccharification, making it promising for commercial-scale lactic acid production (Trakarnpaiboon et al., 2018).

Figure 5. The photograph of PUS-immobilized R. oryzae after 72 h of fermentation (Trakarnpaiboon et al., 2018)

In a different study, Wang et al., (2013) investigated the production of L(+)-lactic acid by *R. oryzae* immobilized in polyvinyl alcohol (PVA) (Figure 6). Modifications included sodium alginate and phosphate esterification to reduce diffusional resistance. The production improved significantly, with maximum production of 106.27 g/L at 38°C, 73.1% yield, and 2.95 g/L.h. The immobilized *R. oryzae* was stable in 14 serial-batch cultures and showed good tolerance to low temperature and long-term storage at 4°C (Wang et al., 2013).

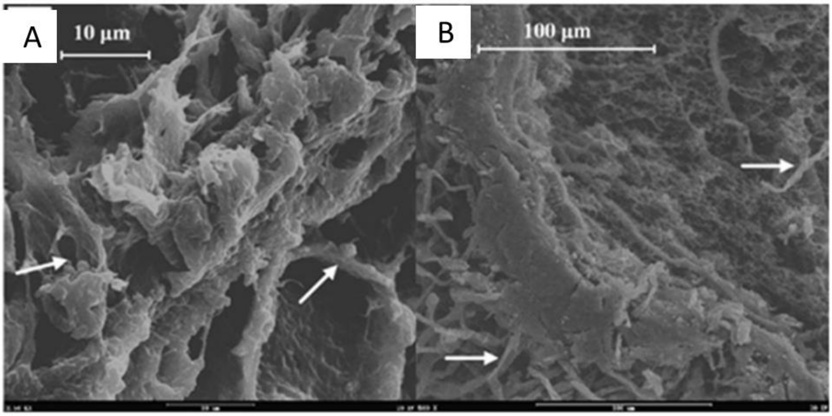

Figure 6. Scanning electron micrographs of PVA beads and immobilized R. oryzae. A) internal structure of a phosphate-modified bead (2,500×), Fungal spores are marked with arrows B) sectional profile of a phosphate-modified bead after fungal proliferation (500×), mycelia are marked with arrows (Wang et al., 2013).

Expanding the application of immobilized *R. oryzae*, a novel immobilized biocatalyst was developed by Efremenko et al. (2006), employing *R. oryzae* fungal cells entrapped in poly(vinyl alcohol)-cryogel (Figure 7). This biocatalyst was evaluated for L(+)-lactic acid production in both batch and semi-batch processes, using some substrates such as glucose and starch. In batch conditions, the biocatalyst achieved LA yields of 94% and 78% from glucose and acid starch hydrolysates, respectively. Semi-batch conditions resulted in product yields of 52% and 45% with the respective substrates. Additionally, the immobilized *R. oryzae* efficiently transformed potato starch (5–70 g L^{-1}) into lactic acid. Importantly, the immobilized biocatalyst demonstrated sustained metabolic activity during long-term operation (at least 480 h) with only a minor decrease (Efremenko et al., 2006).

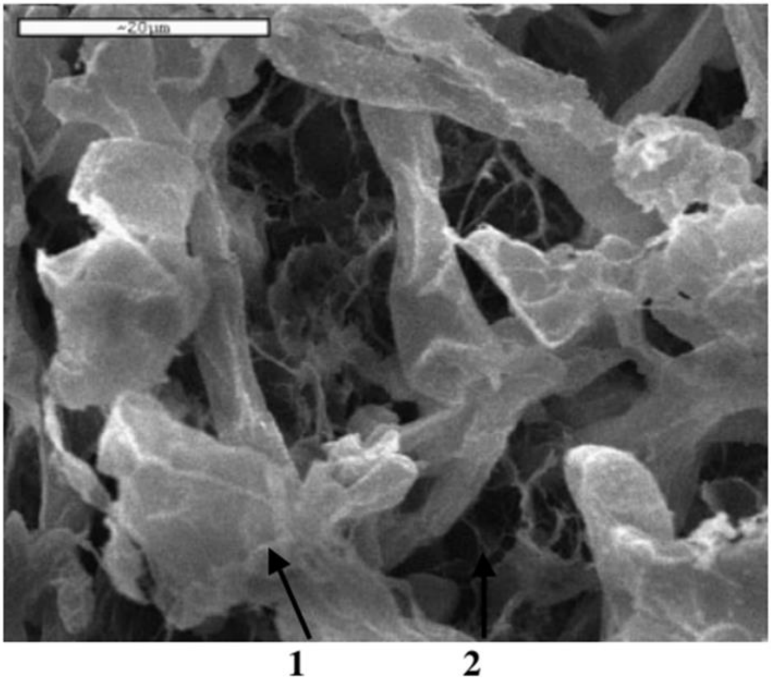

Figure 7. Scanning electron microscopy micrograph of the biocatalyst's surface 1) fungal hyphae, 2) PVA-CG matrix (Efremenko et al., 2006).

In another study, a static bed fermentor was employed for the complete immobilization of *R. oryzae* on a fibrous matrix, leading to improved mass transfer and operational simplicity compared to free cell fermentation in a stirred fermentor. The environmental conditions in this configuration were conducive to the growth of immobilized *R. oryzae*, allowing lactic acid production without substrate repression at high initial glucose concentrations during batch cultivation. The study achieved approximately 67% of the theoretical lactate yield (0.50 g/g) with a productivity of 1.05 g/L h and a final titer of 75.28 g/L in fermentation with a high glucose concentration (150 g/L). Complete cell immobilization enabled *R. oryzae* to produce lactic acid continuously, decreasing cell washout. Continuous growth at an acceptable dilution rate yielded a high concentration of lactic acid (72.32 g/L) with a modest quantity of residual glucose (5 g/L). Immobilized cells in the static bed fermentor revealed remarkable resistance to salt impurities (Na^+, Cl^-) in cassava pulp hydrolysates, which are typically considered toxic to microbial conversion (Pimtong et al., 2017).

Apart from lactic acid production, a net-based immobilization method was developed for fumaric acid fermentation by *R. arrhizus*. The net's large surface area effectively immobilized filamentous mycelia, leading to rapid fumaric acid production (Figure 8). Optimization of net size and spore concentration resulted in improved fermentation performance, with a selected net size of 150 cm^2 and a spore concentration of 0.5 x 10^6 per ml. In comparison to free-cell fermentation, fumaric acid production remained consistent (32.03 vs. 31.23 g/L), but fermentation time was significantly reduced by 83.3% (24 vs. 144 h) (Gu et al., 2013).

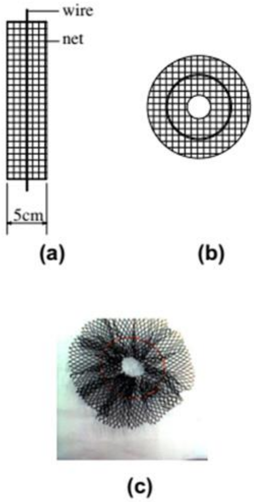

Figure 8. Immobilization device made of wire and fiber net. a) making process b) schematic diagram of device c) net immobilization photo (Gu et al., 2013).

Cao et al. (1996) employed an integrated system combining simultaneous fermentation and adsorption for the production and recovery of fumaric acid from glucose by *R. oryzae*. In this system, *Rhizopus* mycelia were self-immobilized on plastic discs within a rotary biofilm contactor during the nitrogen-rich growth phase. The biofilm, formed during the growth phase, was exposed alternately to a liquid medium and air in the horizontal fermentation vessel during the non-growth, production phase. Fumaric acid, the fermentation product, was continuously and simultaneously removed by a linked adsorption column. This approach helped moderate inhibition, enhance fermentation rate, and sustain cell viability. The removal of fumaric acid also led to the release of hydroxyl ions from a polyvinyl pyridine adsorbent, preventing a pH decrease. The fermentation system demonstrated the capability to produce fumaric acid with an average yield of 85 g/liter from 100 g of glucose per liter within 20 hours under repetitive fed-batch cycles. On a weight yield basis, it achieved 91% of the theoretical maximum with a productivity of 4.25 g/liter/h. In comparison, stirred-tank fermentation supplemented with calcium carbonate achieved an average weight yield of 65% after 72 hours, with a productivity of 0.9 g/liter/h. The immobilized reactor maintained its biological activity without loss during repetitive operation for two weeks (Cao et al., 1996).

Additionally, an innovative approach involving the use of brewery wastewater as a fermentation medium, muslin cloth as an immobilizing device, and the fungal strain *R. oryzae* was employed to enhance fumaric acid production (Figure 9). Optimal conditions were determined as a muslin cloth area of 25 cm^2 and a spore concentration of 1.5×10^6 per mL, resulting in increased fumaric acid production from 30.56 ± 1.40 to 43.67 ± 0.32 g L^{-1} and volumetric productivity from 0.424 to 1.21 g L^{-1} h in immobilized submerged fermentation compared to free-cell fermentation. The specific fumaric acid production rates were comparable between free-cell and muslin cloth immobilization (3.39 and 3.49 g g^{-1} h^{-1}, respectively). Scanning electron microscope studies confirmed the effective attachment of fungal hyphae to the muslin cloths, demonstrating the potential of brewery wastewater and muslin cloth for enhanced fumaric acid production through submerged fermentation (Das et al., 2015).

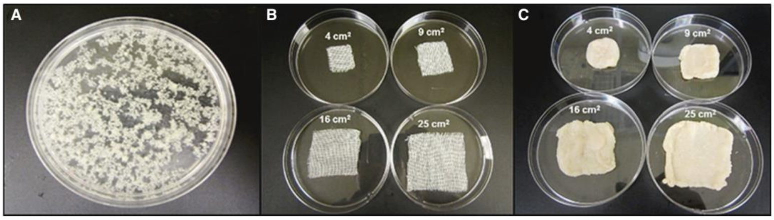

Figure 9. *R. oryzae* cultures A) Pre-cultured cell pellets of *R. oryzae* at 30 °C, 200 rpm for 24 h; B) muslin cloth pieces of different sizes used for immobilization of *R. oryzae*; and C) immobilized mycelium of *R. oryzae* on muslin cloths of different sizes after incubating at 30 °C, 200 rpm for 24 h (Das et al., 2015).

In a related study, the use of immobilized *R. oryzae* for fumaric acid bioproduction using agro-industrial residues as a renewable feedstock was explored. Immobilization with polystyrene foam (Figure 10) was found to be the most suitable method, resulting in a considerable reduction in the lag phase and a 42% improvement in fumaric acid production compared to other support materials. Using apple pomace ultrafiltration sludge as the sole feedstock, the immobilized *R. oryzae* achieved a final fumaric acid titer of 7.9 g/L, outperforming free mycelial fermentation. Intermittent feeding with molasses supplementation further increased fumaric acid production threefold, reaching 5.1 g/L. This approach not only enhances fumaric acid production but also makes the process cost-competitive and environmentally friendly by utilizing renewable feedstocks and reducing the release of greenhouse gases (Sebastian et al., 2021).

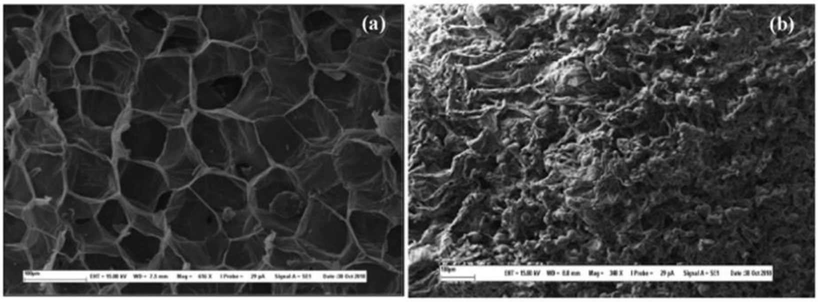

Figure 10. Scanning electron microscopy micrograph of polystyrene beads A) before immobilization B) after fermentation in apple pomace ultrafiltration sludge (Sebastian et al., 2021).

Gallic acid is an organic acid like lactic acid and fumaric acid and has widespread applications. Given its multifaceted properties and versatile applications, it holds considerable significance for both the pharmaceutical and chemical industries. The study investigated the production of gallic acid using immobilized cells of *R. oryzae*. Optimal conditions for maximum gallic acid production included a 2% tannic acid concentration, 200 calcium alginate beads with a spore concentration of 2×10^5/ml, and an initial pH of 5.0. Under these conditions, the tannin conversion rate was 78.5%. In comparison, free cell culture achieved a slightly higher conversion rate of 83.5% within a 4-day incubation period. The immobilized beads demonstrated successful reuse for three cycles. However, treating the beads with glutaraldehyde resulted in a significant decline in the hydrolysis process (Misro et al., 1997).

R. oryzae is a versatile fungus that can also produce lipolytic enzymes. In one study, the potential of *R. oryzae* for producing extracellular lipolytic enzymes with applications in food aroma was investigated through immobilization techniques. Alginate beads were identified as the most suitable carrier material, resulting in the highest lipolytic activities of up to 400 U/L after three days of cultivation. Repeated batch cultures using alginate beads were conducted, demonstrating optimized conditions for consecutive batch fermentations without significant activity loss or deformation, achieving a maximum lipolytic enzyme level of 715 U/L after three batches. The production of lipolytic enzymes by immobilized *R. oryzae* in a 2-liter airlift bioreactor, under optimized conditions, yielded lipolytic activities of 487 U/L. The successive batch operation was smooth, and the bioparticles (fungus growing in alginate beads) maintained their shape throughout fermentation (López et al., 2008).

In another study, *R. arrhizus* cells were immobilized on polyurethane foam for lipase production. The biosynthesis of lipase was found to be repressed at high glucose levels, with maximum productivity observed at a glucose concentration of 1 g/l. The addition of 0.5 g/l corn oil as an inducer in the fermentation medium resulted in a 2.5-fold increase in lipase production compared to the control without oil. Repeated-batch experiments demonstrated that immobilized *R. arrhizus* cells exhibited reproducible behavior, consistently producing the same amount of enzyme over a 120 hours. The storage stability of the enzyme was investigated, showing a 26% reduction in enzyme activity within 160 hours (Elibol & Özer, 2000).

Ban et al. (2001) investigated the use of *R. oryzae* cells, with a 1,3-positional specificity lipase, immobilized within biomass support particles (BSPs) (Figure 11) for the methanolysis of soybean oil, aiming at biodiesel production. The cells easily became immobilized within the BSPs during batch operation. Various substrate-related compounds were tested to enhance methanolysis activity, with olive oil or oleic acid showing significant effectiveness, while glucose was not necessary. Treatment of immobilized cells with organic solvents did not increase activity. Stepwise additions of methanol using BSP-immobilized cells, in the presence of 15% water, resulted in a methyl esters content reaching 90%, demonstrating promise for industrial biodiesel fuel production (Ban et al., 2001).

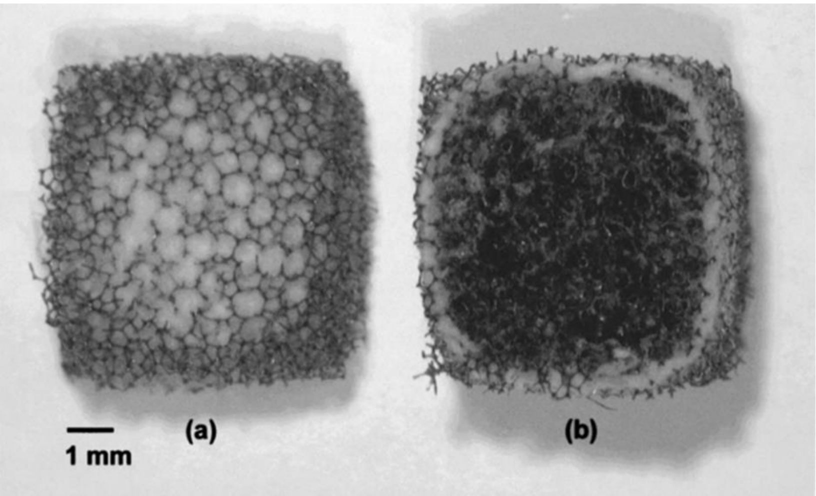

Figure 11. Micrographs of a BSP A) surface B) cross-section (Ban et al., 2001).

R. oryzae is also utilized in the production of polygalacturonase. In a study, highly reactive endo- and exo-polygalacturonases (PGs) were produced using immobilized *R. oryzae* with tobacco industry wastewater. Immobilized cells exhibited a 2.8-fold increase in enzyme activity compared to free cells and reduced production time to 24 hours in shake-flask experiments. The immobilized cells demonstrated the ability for semi-continuous enzyme production through seven consecutive cycles in a scale-up bioreactor. In the first five cycles, average endo-PG and exo-PG activities reached 307.5 and 242.6 U/ml, respectively. The addition of crude enzyme to hydrolyze pectin-containing lignocellulosic biomass under high-gravity conditions resulted in a 4.2-fold increase in glucose release (115.4 vs. 29.0 g/L) compared to hydrolysis using cellulose alone. This process not only efficiently produces pectin-degrading enzymes but also offers a cost-effective method for treating tobacco wastewater and the potential for obtaining high-titer fermentable sugars from pectin-containing lignocellulosic biomass, with implications for commercial biofuel production (Zheng et al., 2016).

Since it is a clean alternative fuel that is renewable, biodegradable, and non-toxic and can typically be used in place of diesel, biodiesel has received a lot of interest. Non-edible *Calophyllum inophyllum* oil is considered a promising feedstock for biodiesel production. A study employed *R. oryzae* cells immobilized on reticulated polyurethane foam for the transesterification of *C. inophyllum* oil. Optimal conditions for processing 9.65 g of *C. inophyllum* oil were determined as follows: methanol to oil molar ratio of 12:1, water content of 15% v/v, cell concentration of 20%, and a temperature of 35°C. In batch methanolysis under these optimized conditions, a high yield of 92% fatty acid methyl esters (FAME) was achieved. Furthermore, in continuous mode with a feed flow rate of 25 l/h, an 87% FAME yield was obtained. The study suggests that the transesterification of *C. inophyllum* oil using *R. oryzae* whole cells immobilized on reticulated polyurethane foam is an efficient process for biodiesel production (Arumugam & Ponnusami, 2014).

There is another study that explores the use of loofah sponge as a biomass carrier for whole-cell immobilization of *R. oryzae* mycelia in biodiesel production. The sponge allows spontaneous attachment of *R. oryzae* cells, reaching a high concentration of 1.40 g per 1 g of LSPs (Figure 12). Investigations on the effects of biocatalyst addition and water content on methanolysis indicate operational stability of the glutaraldehyde-treated biocatalyst at 35 °C, showing a 3.4-fold increase in half-life compared to the untreated biocatalyst. Under optimized conditions, methyl ester yield in the reaction mixture reaches 82.2% to 92.2% in each cycle, highlighting loofah sponge as a potential fungi carrier for an immobilized whole-cell biocatalyst in biodiesel production (He et al., 2016).

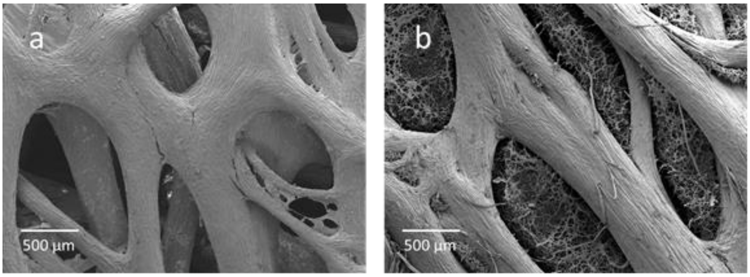

Figure 12. Surface scanning electron microscopy (SEM) of LSPs (a: empty LSP; b: LSP with immobilized dried cells)(He et al., 2016)

Pentachlorophenol (PCP) is an organochlorine compound used as a pesticide and a disinfectant, and extremely toxic to humans from acute ingestion and inhalation exposure. It is shown that the sorption of PCP using nylon fiber with immobilized *R. oryzae* to eliminate the chemical compound is possible. *R. oryzae* was immobilized in nylon fiber cubes, demonstrating growth even in the presence of PCP, which inhibited growth. The fungus removed 88.6% and 92% of PCP in cultures with initial concentrations of 12.5 and 25 mg PCP L^{-1}, respectively, showcasing its potential for PCP removal when immobilized in nylon fiber. This study is the first to report the efficacy of *R. oryzae*, immobilized in nylon fiber for PCP removal (León-Santiestebán et al., 2011).

Azo dyes are the most common and adaptable type of synthetic dyes, and they are widely used in many areas, including food, tannery, printing, cosmetics, paper, textile dyeing, and color photography. Even though they are used extensively, azo dyes are acknowledged as a major class of environmental contaminants. Bagchi et al. (2021) showed the adsorption of the azo dye Reactive Blue 4 (RB4) using both dead and carboxy-methyl cellulose (CMC) immobilized *R. oryzae* as a biosorbent in batch scale. The experiments were carried out in a 250 mL Erlenmeyer flask with a dye concentration ranging from 200 to 500 mg/L in a 50 mL dye solution. Maximum RB4 adsorption capacity (97.44%) was observed under the following conditions: pH 3.0, temperature 30°C, initial dye concentration 200 mg/L, and contact time of 6 hours. Among the tested matrices, CMC was identified as the most effective carrier for immobilizing *R. oryzae* biomass.

4. Conclusion

In conclusion, the studies outlined a diverse range of immobilization techniques and support matrices employed for the cultivation of *Rhizopus* species, particularly *R. oryzae*, to enhance the production of various valuable compounds, including lipolytic enzymes, fumaric acid, biodiesel, LA and gallic acid. The challenges associated with conventional filamentous fungal fermentations, such as the aerobic nature requiring costly aeration and the complexity of controlling cell morphology, have been addressed through innovative immobilization strategies.

The investigation of immobilization methods for LA production with *R. oryzae* revealed the efficacy of methods like gel entrapment using Ca-alginate, polyurethane foam, and loofah sponge. These approaches demonstrated improved fermentation rates, LA transformation efficiency, and stability in repeated batch operations. Moreover, alternative matrices, such as honeycomb-inspired structures and polyvinyl alcohol-cryogel, exhibited enhanced mass transfer and LA production.

In enzyme production, immobilization of *R. oryzae* for lipase and polygalacturonase production using alginate beads, polyurethane foam, and tobacco industry wastewater highlighted the versatility of this fungus in biocatalysis.

For biodiesel production, *R. oryzae* cells which were immobilized on various matrices, including biomass support particles, reticulated polyurethane foam, and loofah sponge, showcased promising results. The studies highlighted the significance of support matrix selection for efficient transesterification of oils into fatty acid methyl esters.

R. oryzae cells immobilized in polyurethane foam, nylon fiber, and muslin fabric showed promise for environmental applications by efficiently eliminating pollutants such as PCP and RB4.

Overall, these studies contribute valuable insights into the application of immobilization techniques and support matrices to enhance the production of various compounds using *Rhizopus* species. The findings not only advance the understanding of fungal fermentation processes but also hold promise for industrial applications, offering sustainable and efficient methods for producing diverse bio-based products.

REFERENCES

Abd Razak, L. D., Abd Rashid, N. Y., Jamaluddin, A., Sharifudin, S. A., Abd Kahar, A., & Long, K. (2017). Cosmeceutical potentials and bioactive compounds of rice bran fermented with single and mix culture of Aspergillus oryzae and Rhizopus oryzae. *Journal of the Saudi Society of Agricultural Sciences*, *16*(2), 127–134.

Arumugam, A., & Ponnusami, V. (2014). Biodiesel production from Calophyllum inophyllum oil using lipase producing Rhizopus oryzae cells immobilized within reticulated foams. *Renewable Energy*, *64*, 276–282. https://doi.org/10.1016/j.renene.2013.11.016

Bagchi, M., Bera, D., & Adhikari, S. (2021). Biosorption of an azo dye Reactive Blue 4 from aqueous solution using dead and CMC immobilized biomass of Rhizopus oryzae (MTCC 262). *Bioremediation Journal*, *25*(4), 326–346. https://doi.org/10.1080/10889868.2021.1884526

Balla, E., Daniilidis, V., Karlioti, G., Kalamas, T., Stefanidou, M., Bikiaris, N. D., Vlachopoulos, A., Koumentakou, I., & Bikiaris, D. N. (2021). *Poly (lactic Acid): A Versatile Biobased Polymer for the Future with Multifunctional Properties — From Monomer Synthesis , Polymerization Techniques and Molecular Weight Increase to PLA Applications*.

Ban, K., Kaieda, M., Matsumoto, T., Kondo, A., & Fukuda, H. (2001). Whole cell biocatalyst for biodiesel fuel production utilizing Rhizopus oryzae cells immobilized within biomass support particles. *Biochemical Engineering Journal*, *8*(1), 39–43. https://doi.org/10.1016/S1369-703X(00)00133-9

Battaglia, E., Benoit, I., Brink, J. Van Den, Wiebenga, A., Coutinho, P. M., Henrissat, B., & Vries, R. P. De. (2011). Carbohydrate-active enzymes from the zygomycete fungus Rhizopus oryzae : a highly specialized approach to carbohydrate degradation depicted at genome level. *BMC Genomics*, *12*(1), 38. https://doi.org/10.1186/1471-2164-12-38

Cao, N., Du, J., Gong, C. S., & Tsao, G. T. (1996). Simultaneous production and recovery of fumaric acid from immobilized Rhizopus oryzae with a rotary biofilm contactor and an adsorption column. *Applied and Environmental Microbiology*, *62*(8), 2926–2931. https://doi.org/10.1128/aem.62.8.2926-2931.1996

Chiou, T. W., Wang, Y. C., & Liu, H. S. (1998). Utilizing the macroporous packed bed for insect cell/baculovirus expression. Part 2: The production of human interleukin-5 in polyurethane foam and cellulose foam packed bed bioreactors. *Bioprocess Engineering*, *18*(2), 91–100.

Das, R. K., Brar, S. K., & Verma, M. (2015). Enhanced fumaric acid production from brewery wastewater by immobilization technique. *Journal of Chemical Technology and Biotechnology*, *90*(8), 1473–1479.

Dotsenko, A. S., Dotsenko, G. S., Senko, O. V., Stepanov, N. A., Lyagin, I. V., Efremenko, E. N., Gusakov, A. V., Zorov, I. N., & Rubtsova, E. A. (2018). Complex effect of lignocellulosic biomass pretreatment with 1-butyl-3-methylimidazolium chloride ionic liquid on various aspects of ethanol and fumaric acid production by immobilized cells within SSF. *Bioresource Technology*, *250*, 429–438.

Efremenko, E., Spiricheva, O., Varfolomeyev, S., & Lozinsky, V. (2006). Rhizopus oryzae fungus cells producing L(+)-lactic acid: Kinetic and metabolic parameters of free and PVA-cryogel-entrapped mycelium. *Applied Microbiology and Biotechnology*, *72*(3), 480–485.

Elibol, M., & Özer, D. (2000). Lipase production by immobilised *Rhizopus arrhizus. Process Biochemistry*, *36*(3), 219–223.

Furusaki, S., & Seki, M. (1992). Use and engineering aspects of immobilized cells in biotechnology. *Adv Biochem Eng Biotechnol.*, *23*, 161–185.

Ganguly, R., Dwivedi, P., & Singh, R. P. (2007). Production of lactic acid with loofa sponge immobilized Rhizopus oryzae RBU2-10. *Bioresource Technology*, *98*(6), 1246–1251. https://doi.org/10.1016/j.biortech.2006.05.004

Ghosh, B., Karmakar, M., & Ray, R. R. (2015). Production of Extracellular Isoamylase and Endoglucanase by Entrapped Spores of Rhizopus oryzae PR7. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*(3), 733–738.

Ghosh, B., & Ray, R. R. (2011). Current commercial perspective of Rhizopus oryzae: A review. *Journal of Applied Sciences*, *11*(14), 2470–2486. https://doi.org/10.3923/jas.2011.2470.2486

Gu, C., Zhou, Y., Liu, L., Tan, T., & Deng, L. (2013). Production of fumaric acid by immobilized Rhizopus arrhizus on net. *Bioresource Technology*, *131*, 303–307. https://doi.org/10.1016/j.biortech.2012.12.148

Hamamci, H., & Ryu, D. D. Y. (1994). Production of L(+)-lactic acid using immobilized rhizopus oryzae reactor performance based on kinetic model and simulation. *Applied Biochemistry and Biotechnology*, *44*(2), 125–133. https://doi.org/10.1007/BF02921650

Hang, Y. D., Hamamcı, H., & Woodams, E. E. (1989). Production of L8+)-Lactic acid by Rhizopus oryzae immobilized in calcium alginate gels. *Biotechnology Letters*, *11*(2), 119–120.

He, Q., Shi, H., Gu, H., Naka, G., Ding, H., Li, X., Zhang, Y., Hu, B., & Wang, F. (2016). Immobilization of *Rhizopus oryzae* LY6 onto Loofah Sponge as a Whole-Cell Biocatalyst for Biodiesel Production. *BioResources*, *11*(1), 850–860.

Katzbauer, B., Narodoslawsky, M., & Moser, A. (1995). Classification system for immobilization techniques. *Bioprocess Engineering*, *12*(4), 173–179. https://doi.org/10.1007/BF01767463

León-Santiestebán, H., Meraz, M., Wrobel, K., & Tomasini, A. (2011). Pentachlorophenol sorption in nylon fiber and removal by immobilized Rhizopus oryzae ENHE. *Journal of Hazardous Materials*, *190*(1–3), 707–712. https://doi.org/10.1016/j.jhazmat.2011.03.101

López, E., Deive, F. J., & Longo, M. A. (2008). *Lipolytic Enzyme Production by Immobilized Rhizopus oryzae*. *11*, 1555–1560.

Lozinsky, V. I. (2008). Polymeric cryogels as a new family of macroporous and supermacroporous materials for biotechnological purposes. *Russian Chemical Bulletin*, *57*(5), 1015–1032.

Lusta, K. A., Chung, I. K., Sul, I. W., Park, H. S., & Shin, D. I. (2000). Immobilization of fungus Aspergillus sp. by a novel cryogel technique for production of extracellular hydrolytic enzymes. *Process Biochemistry*, *35*(10), 1177–1182. https://doi.org/10.1016/S0032-9592(00)00158-8

Maslova, O., Stepanov, N., Senko, O., & Efremenko, E. (2019). Production of various organic acids from different renewable sources by immobilized cells in the regimes of separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SFF). *Bioresource Technology*, *272*(September 2018), 1–9.

Misro, S. K., Kumar, M. R., Banerjee, R., & Bhattacharyya, B. C. (1997). Production of gallic acid by immobilization of Rhizopus oryzae.

Bioprocess Engineering, *16*(5), 257–260.

Mitchell, C. (1926). *Vinegar: its manufacture and examination* (2nd Editio). Griffin.

Naude, A., & Nicol, W. (2017). Fumaric acid fermentation with immobilised Rhizopus oryzae: Quantifying time-dependent variations in catabolic flux. *Process Biochemistry*, *56*, 8–20.

Nursiwi, A., Pertiwi, F., Ishartani, D., Sari, A. M., & Zaman, M. Z. (2021). Substrates and storage time evaluation for preparing tempeh starter from Rhizopus oryzae Substrates and storage time evaluation for preparing tempeh starter from Rhizopus oryzae CBS130145. *IOP Conference Series: Earth and Environmental Science*, *828*(012003), 1–6.

Özer Uyar, G. E., & Uyar, B. (2023). *Potato peel waste fermentation by Rhizopus oryzae to produce lactic acid and ethanol*. *April*, 5908–5917. https://doi.org/10.1002/fsn3.3670

Pimtong, V., Ounaeb, S., Thitiprasert, S., Tolieng, V., Sooksai, S., Boonsombat, R., Tanasupawat, S., Assabumrungrat, S., & Thongchul, N. (2017). Enhanced effectiveness of Rhizopus oryzae by immobilization in a static bed fermentor for L-lactic acid production. *Process Biochemistry*, *52*, 44–52. https://doi.org/10.1016/j.procbio.2016.09.020

Ranjit, C., & Srividya, S. (2016). Lactic acid production from free and Polyurethane immobilized cells of Rhizopus oryzae MTCC 8784 by direct hydrolysis of starch and agro-industrial waste. *International Food Research Journal*, *23*(6), 2646–2652.

Sebastian, J., Dominguez, K. V., Brar, S. K., & Rouissi, T. (2021). Fumaric acid production using alternate fermentation mode by immobilized Rhizopus oryzae-a greener production strategy. *Chemosphere*, *281*(November 2020), 130858. https://doi.org/10.1016/j.chemosphere.2021.130858

Shahri, S. Z., Vahabzadeh, F., & Mogharei, A. (2020). Lactic acid production by loofah-immobilized Rhizopus oryzae through one-step fermentation process using starch substrate. *Bioprocess and Biosystems Engineering*, *43*(2), 333–345. https://doi.org/10.1007/s00449-019-02231-5

Sizemore, S. R., Nichols, R., Tatum, R., Atanassov, P., Johnson, G. R., & Luckarift, H. R. (2013). Immobilization of Whole Cells by Chemical Vapor Deposition of Silica. In J. Guisan (Ed.), *Immobilization of*

Enzymes and Cells. Methods in Molecular Biology (3rd Editio, pp. 301–312). Humana Press, Totowa, NJ.

Sun, Y., Li, Y. L., Yang, H., Bai, S., & Hu, Z. D. (1996). Characteris-tics of immobilized *Rhizopus oryzae* in polyurethane foam cubes. *Biotechnology Techniques*, *10*(11), 809–814.

Tanyıldızı, M. Ş., Bulut, Ş., Selen, V., & Özer, D. (2012). Optimization of lactic acid production with immobilized Rhizopus oryzae. *African Journal of Biotechnology*, *11*(34), 8546–8552. https://doi.org/10.5897/ajb11.1706

Tay, A., & Yang, S. (2002). Production of L (+) -Lactic Acid From Glucose and Starch by Immobilized Cells of Rhizopus oryzae in a Rotating Fibrous Bed Bioreactor. *Biotechnology and Bioengineering*, *80*(1), 1–12. https://doi.org/10.1002/bit.10340

Tosa, T., & Shibatani, T. (1995). Industrial Application of Immobilized Biocatalysts in Japan. *Annals of the New York Academy of Sciences*, *750*(1), 364–375.

Trakarnpaiboon, S., Praneetrattananon, S., & Kitpreechavanich, V. (2018). Simultaneous saccharification and fermentation of L-(+)-lactic acid production from liquefied cassava starch by immobilized rhizopus oryzae in a 3 L airlift fermenter. *Chiang Mai Journal of Science*, *45*(1), 77–91.

Trelles, J. A., & Rivero, C. W. (2020). Whole Cell Entrapment Techniques. In J. Guisan, J. Bolivar, F. López-Gallego, & J. Rocha-Martín (Eds.), *Immobilization of Enzymes and Cells. Methods in Molecular Biology, vol 2100*. Humana, New York, NY. https://doi.org/https://doi.org/10.1007

Virkajärvi, I., & Linko, M. (1999). Immobilization: A revolution in traditional brewing. *Naturwissenschaften*, *86*(3), 112–122.

Wang, P., Chen, Z., Li, J., Wang, L., Gong, G., Zhao, G., Liu, H., & Zheng, Z. (2013). Immobilization of Rhizopus oryzae in a modified polyvinyl alcohol gel for L(+)-lactic acid production. *Annals of Microbiology*, *63*(3), 957–964. https://doi.org/10.1007/s13213-012-0549-x

Wang, Z., Wang, Y., Yang, S. T., Wang, R., & Ren, H. (2010). A novel honeycomb matrix for cell immobilization to enhance lactic acid production by Rhizopus oryzae. *Bioresource Technology, 101*(14), 5557–5564. https://doi.org/10.1016/j.biortech.2010.02.064

Yamane, T., & Tanaka, R. (2013). Highly accumulative production of l(+)-

lactate from glucose by crystallization fermentation with immobilized Rhizopus oryzae. *Journal of Bioscience and Bioengineering*, *115*(1), 90–95. https://doi.org/10.1016/j.jbiosc.2012.08.005

Yen, H. W., & Lee, Y. C. (2010). Production of lactic acid from raw sweet potato powders by rhizopus oryzae immobilized in sodium alginate capsules. *Applied Biochemistry and Biotechnology*, *162*(2), 607–615. https://doi.org/10.1007/s12010-009-8884-5

Zheng, Y., Wang, Y., Zhang, J., & Pan, J. (2016). Using tobacco waste extract in pre-culture medium to improve xylose utilization for l-lactic acid production from cellulosic waste by Rhizopus oryzae. *Bioresource Technology*, *218*, 344–350. https://doi.org/10.1016/j.biortech.2016.06.071

CHAPTER 7

INSIGHTS INTO IMMOBILIZATION TECHNIQUES AND INDUSTRIAL APPLICATIONS OF *Rhizopus oryzae*

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INTRODUCTION

Immobilization plays a pivotal role in the realm of biotechnology, particularly when it comes to enzymes or cells. This chapter discusses the significance of immobilization in different industrial processes, focusing on its application in the synthesis of physiologically active compounds such as organic acids, extracellular enzymes, etc. The use of immobilized microorganisms, particularly in industrial fermentation, has proven to be a breakthrough, enabling continuous processes with significant economic benefits. The examples of the efficacy of immobilized microorganisms in the industrial production of substances like L-aspartic acid, L-malic acid, and semisynthetic penicillin demonstrate the practical significance of this biotechnological technique.

This chapter also provides a glimpse into the diverse applications of *R. oryzae*, with a specific focus on its role in L(+)-lactic acid production and the various immobilization strategies employed to enhance fermentation processes. In the sections that follow, it will be discussed that individual research projects and conclusions about *R. oryzae* immobilization, provide insight into the complexities of these procedures and how they may affect the development of biotechnology.

The examination of immobilization techniques and their uses lays the basis for an in-depth understanding of their function in process optimization and innovation in biotechnological advances.

1. Immobilization

The limitation or prevention of the movement is called immobilization. In the field of biotechnology, when enzymes or cells are immobilized, it indicates that they are affixed to a substrate or one another (Virkajärvi & Linko, 1999). The use of immobilized cells in industrial processes dates back to ancient times. For instance, there is documentation of the immobilization of bacteria for vinegar production dating back to as early as 1670 (Mitchell, 1926).

1.1. The importance of immobilization

The significance of immobilization in biotechnological processes lies in its potential to optimize these processes, leading to increased productivity, higher product concentrations, and improved conversion of raw materials

(Katzbauer et al., 1995). The objective of immobilizing living cells is to create a biocompatible environment that preserves biocatalytic activity while facilitating the flow of essential nutrients. In several instances, encapsulated cells sustain viability without proliferation, ensuring prolonged catalytic activity without a loss of viability (Sizemore et al., 2013). The use of immobilized microorganisms, particularly for producing biologically active substances like extracellular enzymes, has gained attention due to its applicability across various industrial processes (Lusta et al., 2000). Immobilization facilitates continuous processes, offering substantial economic benefits, and has proven to be a breakthrough in some cases where continuous processes with free cells were initially disappointing (Virkajärvi & Linko, 1999). Moreover, the practice of immobilizing microorganisms has typically been appealing in industrial fermentation as a means to enhance the production yield of desired products while allowing for the repeated utilization of the immobilized cells (Tanyıldızı et al., 2012; Trakarnpaiboon et al., 2018).

The success of immobilized microbial cells have been demonstrated in the industrial production of various substances, such as L-aspartic acid, L-malic acid, L-alanine, D-aspartic acid (used in semisynthetic penicillin), high glucose syrup, palatinose, and acrylamide (Tosa & Shibatani, 1995).

Lusta et al. (2000) proposed creating a gel matrix with macroporous structure designed to serve as a suitable support for immobilizing filamentous fungi. The unique features of this gel matrix were focused on regulating porosity and ensuring its capacity to withstand extended periods of repeated batch processing for the production of extracellular cellulases and xylanase (Lusta et al., 2000).

The immobilization of fungal spores in the carrier matrix enables the formation of small granules containing metabolically active mycelium. This arrangement promotes an advantageous distribution of hyphae, facilitating the active synthesis of organic acids (OAs) (Naude & Nicol, 2017; Pimtong et al., 2017). The use of poly(vinyl alcohol) (PVA) cryogel as a carrier for immobilizing filamentous fungal cells has proven to be highly effective (Dotsenko et al., 2018), because this carrier exhibits both high chemical and mechanical stability, along with a well-developed macroporous structure that facilitates mass transfer (Lozinsky, 2008). Another study focuses on the production of various organic acids such as succinic, fumaric, or lactic, by microbial cells such as filamentous fungi *R. oryzae* and bacteria *Actinobacillus succinogenes* immobilized in “PVA cryogel using diverse renewable raw materials (wheat and rice straw, aspen and pine sawdust, Jerusalem artichoke stems and tubers, biomass of macro- and microalgae) in batch conditions”. Compared to free cells, immobilized cells exhibited higher process productivity, bulk output, and concentrations of organic acids under identical conditions (Maslova et al., 2019).

1.2. Methods of cell immobilization

Various methods of cell immobilization are commonly employed in biotechnology (Katzbauer et al., 1995), primarily achieved through entrapment within diverse organic or inorganic matrices or by attaching them to surfaces. Binding methods include physical adsorption or chemical binding, and combinations like adsorption followed by cross-linking (Figure 1). Entrapment techniques, widely utilized for whole-cell immobilization, involve enclosing microorganisms in a porous network through physical or chemical polymerization. This method, known for its simplicity and minimal biocatalyst requirement, starts with suspending microorganisms in a selected matrix solution. When selecting a matrix solution, various properties such as price, cell load, ease and type of immobilization, mass transfer, regeneration, reactor channeling or blocking, stability, sterilization, rigidity, and approval for food use should be considered (Virkajärvi & Linko, 1999). Examples of matrices include cellulose derivatives, diatomaceous earth, porous glass, silicon carbide, silicon rubber, and wood chips. Factors like cost, regeneration necessity, and suitability for food use influence matrix choice. Wood chips, for instance, are a cost-effective matrix for yeast in continuous reactors and may not require regeneration due to their low cost, but regeneration should be simple if needed.

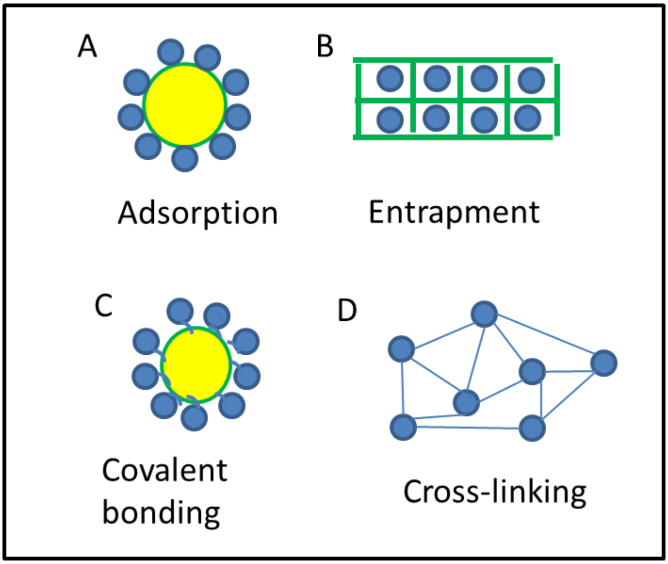

Figure 1. Immobilization methods: (A) Adsorption, (B) Entrapment, (C) Covalent binding, (D) Cross-linking.

Matrices in entrapment methods include synthetic polymers (polyvinyl alcohol, polyacrylamide, polyurethane), thermogels (cellulose, agarose, agar), and hydrogels (chitosan, alginate, κ-carrageenan). Sodium alginate entrapment is a well-known method where cells mixed with water-soluble alginate forms water-insoluble alginate beads in a calcium chloride solution. Agar or agarose entrapment follows a similar procedure in an oil solution. Another common synthetic method employs polyacrylamide gel, involving the polymerization of acrylamide monomer, whole cells, and a cross-linker reagent (Trelles & Rivero, 2020). However, isotropic xerogel carriers, while widely used, have drawbacks such as low durability: decreased microorganism activity due to matrix formation, requiring optimization for each microorganism and mass transfer issues, along with inducing stress and toxicity in immobilized cells (Lusta et al., 2000). Techniques to address mass transfer resistance include immobilizing cells in thin films, attaching them to the surface of membranes, or using a fibrous matrix achieved through natural attachment or crosslinking to fibers. Although there are difficulties in matching the size and form of the cells, macroporous sponge carriers such as polyurethane (Trakarnpaiboon et al., 2018) or cellulose foams (Chiou et al., 1998) have been used to address these problems.

1.3. Advantages of Immobilization

Immobilization is favored for enhancing process efficiency by addressing challenges associated with submerged fermentation, including aggregate formation and microbial growth inhibition due to metabolite accumulation (López et al., 2008). Immobilized whole cells offer advantages compared to ordinary suspension culture systems, such as avoiding cell wash-out at high dilution rates, achieving higher cell concentrations in the reactor, facilitating separation of cells from product-containing solution or the system, the ability to reuse biocatalysts, reduced susceptibility of cells to contamination, improved process control, and high volumetric productivity (Furusaki & Seki, 1992; Yen & Lee, 2010).

2. *Rhizopus oryzae* and its Industrial Uses

The genus *Rhizopus* belongs to the Phylum Zygomycota, class Zygomycete, order Mucorales, family Mucoraceae, and genus *Rhizopus. Rhizopus* is characterized by the formation of rhizoids (Figure 2), and while some strains are found in plants or animals, *R. oryzae*, a species within this genus, is remarkable for efficiently producing various bioproducts. It is utilized in the production of traditional food items like tempeh, peka, ragi, and loog-pang, organic acids (lactic acid (LA), fumaric acid, gallic acid), alcoholic beverages, and enzymes such as cellulases, tannases, glucoamylases and polygalacturonases (Abd Razak et al., 2017; Ghosh & Ray, 2011; Nursiwi et al., 2021). Due to its commercial significance, *R. oryzae* is considered a microorganism of great interest.

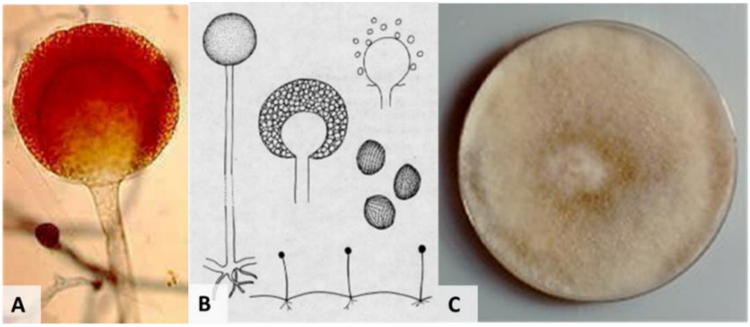

Figure 2. *R. oryzae,* A) The microscopic appearance of sporangia, B) Illustrative scheme of hyphae, columella, and rhizoids C) The colonial appearance

R. oryzae is widely distributed in nature, particularly in tropical and subtropical regions, and is commonly isolated from diverse substrates, including soils, decomposing vegetables, fruits, and seeds. Its rapid growth makes it a primary or secondary colonizer, quickly invading digestible substrates rich in simple sugars (Battaglia et al., 2011). *R. oryzae* is an industrially employed microorganism, because it is recognized as generally safe (GRAS), and is able to utilize a wide range of carbon sources (Ghosh & Ray, 2011).

Moreover, *R. oryzae* is extensively researched for its commercial potential in producing L(+)-lactic acid. In basic media with minimal nutrient supplementation, it is capable of producing optically pure L-(+)-lactic acid. It offers advantages over lactic acid bacteria (LAB) due to lower nutrient requirements, and its product is often optically pure, unlike the racemic mixture produced by LAB. Compared to commonly used bacterial producers, *R. oryzae* can tolerate higher concentrations of accumulated LA. Additionally, *R. oryzae*'s amylolytic ability allows it to produce lactic acid from starchy materials without the need for glucoamylase during fermentation, potentially reducing enzyme costs significantly (Battaglia et al., 2011; Özer Uyar & Uyar, 2023). Currently, there is a growing demand for L-(+)-lactic acid, particularly for the production of the biopolymer polylactic acid (PLA). PLA represents a promising alternative to petrochemical-derived plastics, offering biodegradability, biocompatibility, and environmental friendliness. PLA finds applications in various fields, including disposable consumer products, surgical sutures, drug delivery systems, and orthopedic implants (Balla et al., 2021).

3. Immobilization of *R. oryzae*

LA fermentation by *R. oryzae* has some drawbacks, such as the aerobic nature requiring the cost of aeration (bioreactor agitation and/or air supply). The fungus exhibits different morphologies (filamentous cells, clumps, pulpy structures, flocs, and pellets) influenced by factors like temperature, agitation rate, pH, and medium composition. Filamentous fungi fermentation faces complications like reduced oxygen transfer, wall growth, increased broth viscosity due to mycelial growth, which are critical for LA production. Numerous techniques have been investigated to control cell density and morphology and enhance product yield, production rate, and productivity

(Yamane & Tanaka, 2013). Tay and Yang (2002) summarized these methods, including self-immobilization by forming pellets, cell entrapment within polymeric matrices, cell attachment via surface adsorption. Immobilization simplifies bioreactor operation and control, aids in separating culture media from fungal cells, and facilitates the reutilization of fungal cells to enable long-term LA production (Tay & Yang, 2002).

Researchers have explored various immobilization methods for LA production with *R. oryzae*, with entrapment methods, particularly using soft gels like Ca-alginate, being commonly employed in some studies (Ghosh et al., 2015; Hang et al., 1989; López et al., 2008). However, challenges related to oxygen supply limitation in gel-entrapping methods may reduce fermentation rates and L(+)-lactic acid transformation efficiency. Cell immobilization on a support polymer matrix has been proposed to overcome problems which are related to fermentations with filamentous fungi (Ranjit & Srividya, 2016).

Additionally, previous studies with *Rhizopus* species in submerged cultures explored the effects of ion concentration on lactic acid production, the stability of immobilization in repeated batch fermentations, and the possibility of producing valuable chemicals alongside LA.

Various studies and reviews also discussed the literature on organic acid production using immobilized *R. oryzae* and potential immobilization matrices for LA production.

For example, a study investigated L(+)-lactic acid production using *R. oryzae* immobilized on alginate in a tapered-column fluidized-bed batch reactor. Saturation kinetics with substrate and product inhibitions were observed in linear form, and kinetic constants were determined: V_m (maximal rate) was 11.04 g lactic acid/(L-bead·h), K_{in} (substrate inhibition constant) was 20.9 g glucose/L, K_i (product inhibition constant) was 365 g glucose/L, and K_p (product inhibition constant for lactic acid) was 316 g lactic acid/L. Simulation results aligned well with experimental data when considering the initial lag phase in the model. 73 g/L of L(+)-lactic acid was obtained after 45 h, when initial glucose concentration was 150 g/L. Based on the amount of glucose consumed, with a yield of lactic acid was calculated to be 65% (Hamamci & Ryu, 1994).

Following this, Yen and Lee (2010) utilized *R. oryzae* immobilized in calcium alginate for lactic acid fermentation, unhydrolyzed raw sweet potato powders was used as the primary carbon source. The effects of calcium chloride concentration, immobilized bead diameter, and sodium alginate concentration on lactic acid generation were examined in this study. Higher sodium alginate concentrations during gelation resulted in a more rigid immobilized capsule, leading to reduced lactic acid production. Increased calcium chloride concentrations thickened the immobilized capsule, causing higher mass transfer resistance, but effects were not significant below 15%. Larger bead diameters provided more space for cell growth, optimizing LA production, with the maximum observed at a 5-mm diameter. Repeated batch operations demonstrated that immobilized cells exhibited greater stability in LA production compared to free cells. In comparison to free-cell operation, the total cumulative lactic acid generation in seven repeated batches (216 hours) increased by 55% in immobilized-cell operation, with no noticeable amylolytic activity loss. The findings suggest that *R. oryzae* immobilized in Ca-alginate holds promise for efficient LA production from unhydrolyzed raw sweet potato powders (Yen & Lee, 2010).

In order to produce a significant amount of LA from glucose, *R. oryzae* was cultured in both batch and fed-batch modes. In a three-baffled shaking flask at 37°C, the mycelia of *R. oryzae* were trapped *in situ* within sponge-like cubic particles. Fine powdery calcium carbonate ($CaCO_3$) was added either initially or intermittently to control the pH of the culture liquids. High LA accumulations of 145 g/L and 231 g/L were achieved in batch and fed-batch cultures, respectively, with glucose concentrations exceeding 150 g/L. "In these cultures, the yields and productivities of LA were 95.0%, 1.42 g/L.h, and 92.5%, 1.83 g/L.h, respectively" (Yamane & Tanaka, 2013).

Continuing the exploration of alternative matrices, a loofah sponge emerged as a promising candidate for LA production by immobilized *R. oryzae* (Figure 3). The loofah sponge kept its form and texture even after being treated with buffers of various pH and autoclaved for up to four cycles. The optimal conditions for LA production were found to be a medium containing four pieces of loofa sponge per 100 ml, inoculated with 3×10^6 spores ml^{-1}, resulting in a maximum LA production of 81 g L^{-1} in 48 hours of fermentation. Batch fermentation was successfully repeated ten times, achieving remarkably higher productivities (1.7–1.8 g L^{-1} h^{-1}) during the first five batches, with the peak productivity (1.8 g L^{-1} h^{-1}) observed in the third fermentation batch (Ganguly et al., 2007).

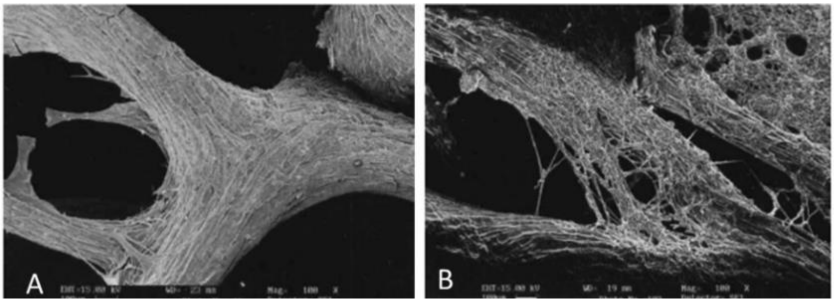

Figure 3. Scanning electron micrograph of R. oryzae immobilized in the loofah sponge; A), Bare loofah sponge; B) Loofah sponge immobilized with R. oryzae (Ganguly et al., 2007).

Expanding on the utilization of loofah sponge, *R. oryzae* demonstrated its proficiency in synthesizing LA from 10 g/l of soluble potato starch through a single-step fermentation process. Immobilizing *R. oryzae* (LIRO) was used to test the natural capacity of fungus to produce biofilms on a loofah sponge (Figure 4). LIRO achieved a maximum LA concentration of 4 g/l within 96 hours, outperforming free cells, which reached 3 g/l. LIRO also exhibited higher specific LA formation and specific starch utilization rate compared to free cells. In a column reactor study using a cell immobilization strategy with optimized temperature and inoculum size, LIRO produced a maximum of 5 g/l of LA within 48 hours in an airlift reactor with a net draft tube (Shahri et al., 2020).

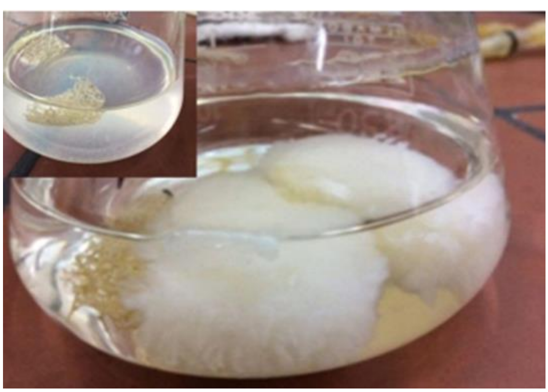

Figure 4. Photograph of the LIRO cells, small photograph shows bare loofah (Shahri et al., 2020).

Wang et al. (2010) developed a novel honeycomb-inspired support matrix for the immobilization of *R. oryzae* cells, aiming to control fungal morphology and improve mass transfer in a bioreactor for lactic acid production. The matrix, featuring asterisk-shaped fibrous structures in a honeycomb configuration, offered a large surface area for cell attachment and biofilm growth. Remarkably, over 90% of inoculated spores were adsorbed onto the matrices within 6–8 hours, achieving 100% immobilization efficiency after 10 hours. In shake-flask experiments with 80 g/L initial glucose, lactic acid production increased by 70% (49.5 g/L vs. 29.3 g/L), and fermentation time decreased by 33% (48 hours vs. 72 hours) compared to free-cell fermentation. The immobilized-cell fermentation was further assessed in a bubble-column bioreactor operated in a repeated batch mode for nine cycles over 36 days. The highest lactic acid production reached 68.8 g/L, with a volumetric productivity of 0.72 g/L h and a lactic acid yield of 93.4% (w/w) from consumed glucose. The overall yield and productivity were 77.6% and 0.57 g/L h, respectively, suggesting the potential for further improvement by optimizing aeration and mixing in the bubble-column bioreactor (Wang et al., 2010).

As another alternative matrix, the researchers focused on the fermentation behavior of *R. oryzae* immobilized in polyurethane foam cubes of various sizes. Through the natural attachment method, *R. oryzae* could be easily immobilized on polyurethane foam cubes larger than 2.5 x 5 x 5 mm^3. The use of small cubes for immobilization proved highly effective in enhancing the productivity of the immobilized cells. However, challenges were encountered in achieving complete immobilization of *R. oryzae* in small cubes, even though increasing the inoculum size improved the immobilization ratio. Microscopic observations revealed that the pore size of the cube matrices was in the range of 0.2 to 0.5 mm. The large pore size did not effectively restrict mycelial growth within the matrices of very small cubes. The authors suggest that a polyurethane foam with smaller pores would contribute to complete immobilization in very small cubes, and efforts are underway to find such a foam to validate this hypothesis (Sun et al., 1996).

Lactic acid production by *R. oryzae* immobilized in polyurethane foam was investigated using response surface methodology with a 23 full-factorial central composite design involving three independent variables: glucose concentration, pH, and agitation rate. The model F-value of 17.01 indicates a good fit. The linear and quadratic effects of glucose concentration and the quadratic effect of agitation rate were found to be significant for lactic acid production. The maximum lactic acid production of 93.2 g/L was achieved with a glucose concentration of 150 g/L, pH 6.39, and agitation rate of 147 rpm. Glucose concentration and agitation rate were identified as limiting parameters, showing that small variations in these parameters could significantly impact lactic acid production. The initial pH did not affect on lactic acid production due to the presence of a neutralizing agent. Lactic acid production from immobilized whole cells under optimal conditions was approximately 55%, higher than that from suspension culture systems (Tanyıldızı et al., 2012).

Ranjit and Srividya (2016) focused on producing L-lactic acid (LA) from agro-industrial waste using both free and polyurethane-immobilized *R. oryzae*, utilizing the natural hydrolytic capabilities of the fungus. The research compares fungal biomass and lactic acid production from various agro wastes, subjected to three different pre-treatments, with the results obtained from starch. Acid-hydrolyzed sugar bagasse demonstrated the highest lactic acid yield at 79.2 g/L, with a peak volumetric productivity of 1.1 g/L/h. Cultivating polyurethane sponge (PUS)-immobilized *R. oryzae* on starch showed a 1.15-fold increase in LA concentration compared to free cells under specific conditions (30°C, 160 rpm agitation rate, pH 6.0 for 72 h). Successful lactic acid production from starch was achieved through four repeated batch cultures with stable efficiency. Across all tested agro waste substrates, PU-immobilized *R. oryzae* exhibited higher lactic acid yields compared to free cells, with sugar bagasse showing a maximum yield of 88.2 g/L (1.22 times higher than with free cells) under specified conditions (30°C, 160 rpm agitation, pH 6.0 for 72 h).

Trakarnpaiboon et al. (2018) produced L-(+)-lactic acid through the fermentation of starchy material, specifically α-amylase-treated liquefied cassava starch and highlighted for cost reduction and increased value of agricultural products. *R. oryzae* was chosen for its ability to produce the highest amount of L-(+)-lactic acid under these conditions. Shaking flask experiments revealed the significance of KH_2PO_4 and $ZnSO_4.7H_2O$, with 150 g/L of α-amylase-treated liquefied cassava starch maximizing L-(+)-lactic acid production. Immobilizing *R. oryzae* on a $0.25 \times 0.25 \times 0.25$ cm^3 PUS in a 3 L airlift fermenter resulted in L-(+)-lactic acid production of 83.7 g/L with a productivity of 1.16 g/L/h after 72 hours. Repeated batch culture with PUS-immobilized *R. oryzae* succeeded through 4 cycles, maintaining high L-(+)-lactic acid concentration and product yield at 77.7 g/L and 0.62, respectively. Additionally, the study demonstrated the feasibility of achieving L-(+)-lactic acid production in a single stage from liquefied cassava starch without a saccharification process, using *R. oryzae* with glucoamylase activity. Under similar conditions, immobilizing *R. oryzae* on 0.25 × 0.25 × 0.25 cm^3 PUS (Figure 5) led to a high L-(+)-lactic acid production of 83.7 g/L with a productivity of 1.16 g/L/h. The successful repeated-batch culture with PUS-immobilized *R. oryzae* after four cycles, particularly when using α-amylase-treated liquefied cassava starch as a substrate, suggested potential cost reduction in enzyme usage for saccharification, making it promising for commercial-scale lactic acid production (Trakarnpaiboon et al., 2018).

Figure 5. The photograph of PUS-immobilized R. oryzae after 72 h of fermentation (Trakarnpaiboon et al., 2018)

In a different study, Wang et al., (2013) investigated the production of L(+)-lactic acid by *R. oryzae* immobilized in polyvinyl alcohol (PVA) (Figure 6). Modifications included sodium alginate and phosphate esterification to reduce diffusional resistance. The production improved significantly, with maximum production of 106.27 g/L at 38°C, 73.1% yield, and 2.95 g/L.h. The immobilized *R. oryzae* was stable in 14 serial-batch cultures and showed good tolerance to low temperature and long-term storage at 4°C (Wang et al., 2013).

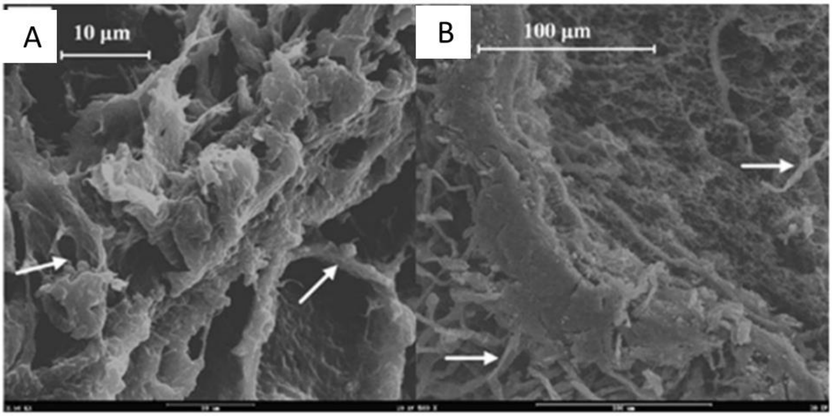

Figure 6. Scanning electron micrographs of PVA beads and immobilized R. oryzae. A) internal structure of a phosphate-modified bead (2,500×), Fungal spores are marked with arrows B) sectional profile of a phosphate-modified bead after fungal proliferation (500×), mycelia are marked with arrows (Wang et al., 2013).

Expanding the application of immobilized *R. oryzae*, a novel immobilized biocatalyst was developed by Efremenko et al. (2006), employing *R. oryzae* fungal cells entrapped in poly(vinyl alcohol)-cryogel (Figure 7). This biocatalyst was evaluated for L(+)-lactic acid production in both batch and semi-batch processes, using some substrates such as glucose and starch. In batch conditions, the biocatalyst achieved LA yields of 94% and 78% from glucose and acid starch hydrolysates, respectively. Semi-batch conditions resulted in product yields of 52% and 45% with the respective substrates. Additionally, the immobilized *R. oryzae* efficiently transformed potato starch (5–70 g L^{-1}) into lactic acid. Importantly, the immobilized biocatalyst demonstrated sustained metabolic activity during long-term operation (at least 480 h) with only a minor decrease (Efremenko et al., 2006).

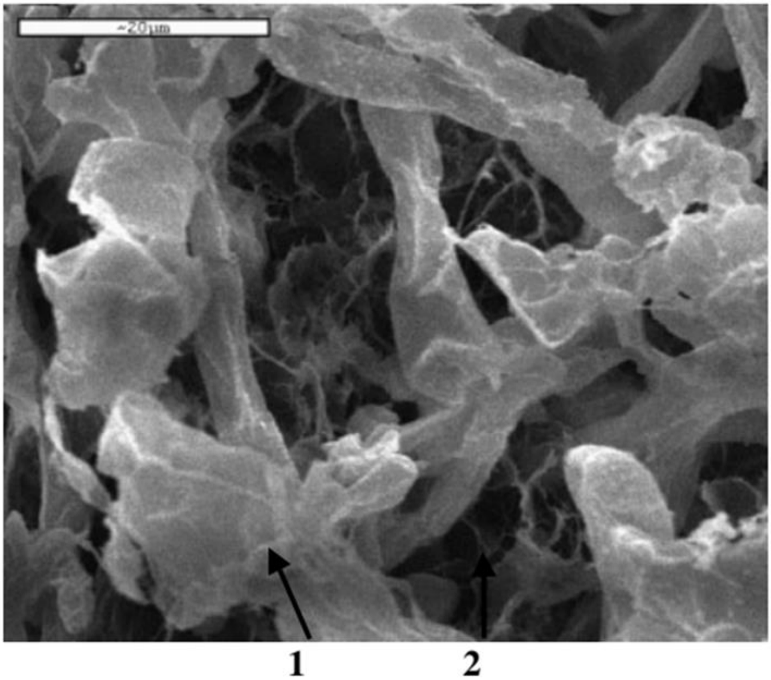

Figure 7. Scanning electron microscopy micrograph of the biocatalyst's surface 1) fungal hyphae, 2) PVA-CG matrix (Efremenko et al., 2006).

In another study, a static bed fermentor was employed for the complete immobilization of *R. oryzae* on a fibrous matrix, leading to improved mass transfer and operational simplicity compared to free cell fermentation in a stirred fermentor. The environmental conditions in this configuration were conducive to the growth of immobilized *R. oryzae*, allowing lactic acid production without substrate repression at high initial glucose concentrations during batch cultivation. The study achieved approximately 67% of the theoretical lactate yield (0.50 g/g) with a productivity of 1.05 g/L h and a final titer of 75.28 g/L in fermentation with a high glucose concentration (150 g/L). Complete cell immobilization enabled *R. oryzae* to produce lactic acid continuously, decreasing cell washout. Continuous growth at an acceptable dilution rate yielded a high concentration of lactic acid (72.32 g/L) with a modest quantity of residual glucose (5 g/L). Immobilized cells in the static bed fermentor revealed remarkable resistance to salt impurities (Na^+, Cl^-) in cassava pulp hydrolysates, which are typically considered toxic to microbial conversion (Pimtong et al., 2017).

Apart from lactic acid production, a net-based immobilization method was developed for fumaric acid fermentation by *R. arrhizus*. The net's large surface area effectively immobilized filamentous mycelia, leading to rapid fumaric acid production (Figure 8). Optimization of net size and spore concentration resulted in improved fermentation performance, with a selected net size of 150 cm^2 and a spore concentration of 0.5×10^6 per ml. In comparison to free-cell fermentation, fumaric acid production remained consistent (32.03 vs. 31.23 g/L), but fermentation time was significantly reduced by 83.3% (24 vs. 144 h) (Gu et al., 2013).

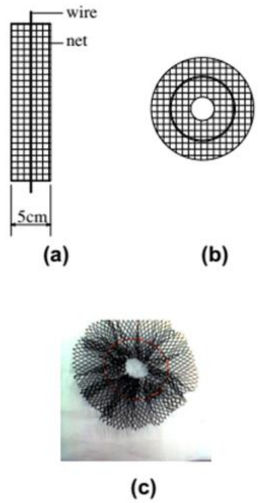

Figure 8. Immobilization device made of wire and fiber net. a) making process b) schematic diagram of device c) net immobilization photo (Gu et al., 2013).

Cao et al. (1996) employed an integrated system combining simultaneous fermentation and adsorption for the production and recovery of fumaric acid from glucose by *R. oryzae*. In this system, *Rhizopus* mycelia were self-immobilized on plastic discs within a rotary biofilm contactor during the nitrogen-rich growth phase. The biofilm, formed during the growth phase, was exposed alternately to a liquid medium and air in the horizontal fermentation vessel during the non-growth, production phase. Fumaric acid, the fermentation product, was continuously and simultaneously removed by a linked adsorption column. This approach helped moderate inhibition, enhance fermentation rate, and sustain cell viability. The removal of fumaric acid also led to the release of hydroxyl ions from a polyvinyl pyridine adsorbent, preventing a pH decrease.

The fermentation system demonstrated the capability to produce fumaric acid with an average yield of 85 g/liter from 100 g of glucose per liter within 20 hours under repetitive fed-batch cycles. On a weight yield basis, it achieved 91% of the theoretical maximum with a productivity of 4.25 g/liter/h. In comparison, stirred-tank fermentation supplemented with calcium carbonate achieved an average weight yield of 65% after 72 hours, with a productivity of 0.9 g/liter/h. The immobilized reactor maintained its biological activity without loss during repetitive operation for two weeks (Cao et al., 1996).

Additionally, an innovative approach involving the use of brewery wastewater as a fermentation medium, muslin cloth as an immobilizing device, and the fungal strain *R. oryzae* was employed to enhance fumaric acid production (Figure 9). Optimal conditions were determined as a muslin cloth area of 25 cm^2 and a spore concentration of 1.5×10^6 per mL, resulting in increased fumaric acid production from 30.56 ± 1.40 to 43.67 ± 0.32 g L^{-1} and volumetric productivity from 0.424 to 1.21 g L^{-1} h in immobilized submerged fermentation compared to free-cell fermentation. The specific fumaric acid production rates were comparable between free-cell and muslin cloth immobilization (3.39 and 3.49 g g^{-1} h^{-1}, respectively). Scanning electron microscope studies confirmed the effective attachment of fungal hyphae to the muslin cloths, demonstrating the potential of brewery wastewater and muslin cloth for enhanced fumaric acid production through submerged fermentation (Das et al., 2015).

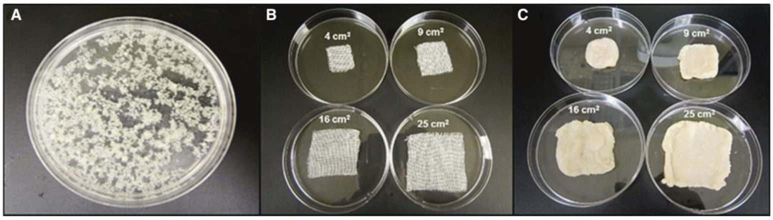

Figure 9. *R. oryzae* cultures A) Pre-cultured cell pellets of *R. oryzae* at 30 °C, 200 rpm for 24 h; B) muslin cloth pieces of different sizes used for immobilization of *R. oryzae*; and C) immobilized mycelium of *R. oryzae* on muslin cloths of different sizes after incubating at 30 °C, 200 rpm for 24 h (Das et al., 2015).

In a related study, the use of immobilized *R. oryzae* for fumaric acid bioproduction using agro-industrial residues as a renewable feedstock was explored. Immobilization with polystyrene foam (Figure 10) was found to be the most suitable method, resulting in a considerable reduction in the lag phase and a 42% improvement in fumaric acid production compared to other support materials. Using apple pomace ultrafiltration sludge as the sole feedstock, the immobilized *R. oryzae* achieved a final fumaric acid titer of 7.9 g/L, outperforming free mycelial fermentation. Intermittent feeding with molasses supplementation further increased fumaric acid production threefold, reaching 5.1 g/L. This approach not only enhances fumaric acid production but also makes the process cost-competitive and environmentally friendly by utilizing renewable feedstocks and reducing the release of greenhouse gases (Sebastian et al., 2021).

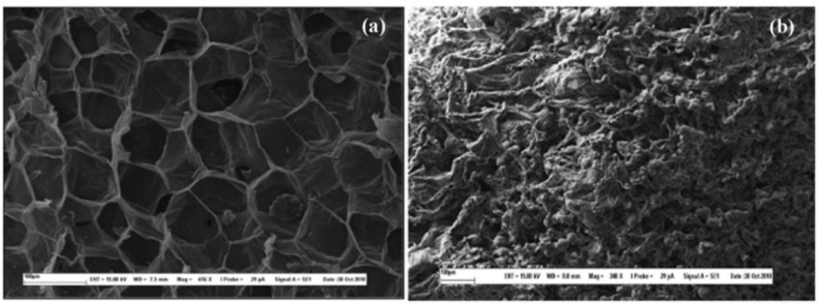

Figure 10. Scanning electron microscopy micrograph of polystyrene beads A) before immobilization B) after fermentation in apple pomace ultrafiltration sludge (Sebastian et al., 2021).

Gallic acid is an organic acid like lactic acid and fumaric acid and has widespread applications. Given its multifaceted properties and versatile applications, it holds considerable significance for both the pharmaceutical and chemical industries. The study investigated the production of gallic acid using immobilized cells of *R. oryzae*. Optimal conditions for maximum gallic acid production included a 2% tannic acid concentration, 200 calcium alginate beads with a spore concentration of 2×10^5/ml, and an initial pH of 5.0. Under these conditions, the tannin conversion rate was 78.5%. In comparison, free cell culture achieved a slightly higher conversion rate of 83.5% within a 4-day incubation period. The immobilized beads demonstrated successful reuse for three cycles. However, treating the beads with glutaraldehyde resulted in a significant decline in the hydrolysis process (Misro et al., 1997).

R. oryzae is a versatile fungus that can also produce lipolytic enzymes. In one study, the potential of *R. oryzae* for producing extracellular lipolytic enzymes with applications in food aroma was investigated through immobilization techniques. Alginate beads were identified as the most suitable carrier material, resulting in the highest lipolytic activities of up to 400 U/L after three days of cultivation. Repeated batch cultures using alginate beads were conducted, demonstrating optimized conditions for consecutive batch fermentations without significant activity loss or deformation, achieving a maximum lipolytic enzyme level of 715 U/L after three batches. The production of lipolytic enzymes by immobilized *R. oryzae* in a 2-liter airlift bioreactor, under optimized conditions, yielded lipolytic activities of 487 U/L. The successive batch operation was smooth, and the bioparticles (fungus growing in alginate beads) maintained their shape throughout fermentation (López et al., 2008).

In another study, *R. arrhizus* cells were immobilized on polyurethane foam for lipase production. The biosynthesis of lipase was found to be repressed at high glucose levels, with maximum productivity observed at a glucose concentration of 1 g/l. The addition of 0.5 g/l corn oil as an inducer in the fermentation medium resulted in a 2.5-fold increase in lipase production compared to the control without oil. Repeated-batch experiments demonstrated that immobilized *R. arrhizus* cells exhibited reproducible behavior, consistently producing the same amount of enzyme over a 120 hours. The storage stability of the enzyme was investigated, showing a 26% reduction in enzyme activity within 160 hours (Elibol & Özer, 2000).

Ban et al. (2001) investigated the use of *R. oryzae* cells, with a 1,3-positional specificity lipase, immobilized within biomass support particles (BSPs) (Figure 11) for the methanolysis of soybean oil, aiming at biodiesel production. The cells easily became immobilized within the BSPs during batch operation. Various substrate-related compounds were tested to enhance methanolysis activity, with olive oil or oleic acid showing significant effectiveness, while glucose was not necessary. Treatment of immobilized cells with organic solvents did not increase activity. Stepwise additions of methanol using BSP-immobilized cells, in the presence of 15% water, resulted in a methyl esters content reaching 90%, demonstrating promise for industrial biodiesel fuel production (Ban et al., 2001).

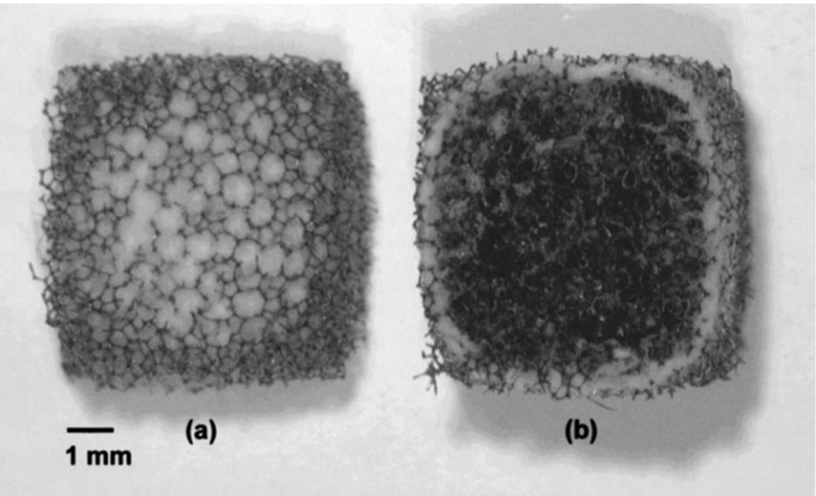

Figure 11. Micrographs of a BSP A) surface B) cross-section (Ban et al., 2001).

R. oryzae is also utilized in the production of polygalacturonase. In a study, highly reactive endo- and exo-polygalacturonases (PGs) were produced using immobilized *R. oryzae* with tobacco industry wastewater. Immobilized cells exhibited a 2.8-fold increase in enzyme activity compared to free cells and reduced production time to 24 hours in shake-flask experiments. The immobilized cells demonstrated the ability for semi-continuous enzyme production through seven consecutive cycles in a scale-up bioreactor. In the first five cycles, average endo-PG and exo-PG activities reached 307.5 and 242.6 U/ml, respectively. The addition of crude enzyme to hydrolyze pectin-containing lignocellulosic biomass under high-gravity conditions resulted in a 4.2-fold increase in glucose release (115.4 vs. 29.0 g/L) compared to hydrolysis using cellulose alone. This process not only efficiently produces pectin-degrading enzymes but also offers a cost-effective method for treating tobacco wastewater and the potential for obtaining high-titer fermentable sugars from pectin-containing lignocellulosic biomass, with implications for commercial biofuel production (Zheng et al., 2016).

Since it is a clean alternative fuel that is renewable, biodegradable, and non-toxic and can typically be used in place of diesel, biodiesel has received a lot of interest. Non-edible *Calophyllum inophyllum* oil is considered a promising feedstock for biodiesel production. A study employed *R. oryzae* cells immobilized on reticulated polyurethane foam for the transesterification of *C.*

inophyllum oil. Optimal conditions for processing 9.65 g of *C. inophyllum* oil were determined as follows: methanol to oil molar ratio of 12:1, water content of 15% v/v, cell concentration of 20%, and a temperature of 35°C. In batch methanolysis under these optimized conditions, a high yield of 92% fatty acid methyl esters (FAME) was achieved. Furthermore, in continuous mode with a feed flow rate of 25 l/h, an 87% FAME yield was obtained. The study suggests that the transesterification of *C. inophyllum* oil using *R. oryzae* whole cells immobilized on reticulated polyurethane foam is an efficient process for biodiesel production (Arumugam & Ponnusami, 2014).

There is another study that explores the use of loofah sponge as a biomass carrier for whole-cell immobilization of *R. oryzae* mycelia in biodiesel production. The sponge allows spontaneous attachment of *R. oryzae* cells, reaching a high concentration of 1.40 g per 1 g of LSPs (Figure 12). Investigations on the effects of biocatalyst addition and water content on methanolysis indicate operational stability of the glutaraldehyde-treated biocatalyst at 35 °C, showing a 3.4-fold increase in half-life compared to the untreated biocatalyst. Under optimized conditions, methyl ester yield in the reaction mixture reaches 82.2% to 92.2% in each cycle, highlighting loofah sponge as a potential fungi carrier for an immobilized whole-cell biocatalyst in biodiesel production (He et al., 2016).

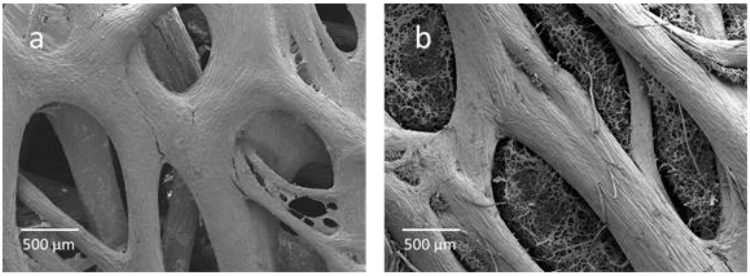

Figure 12. Surface scanning electron microscopy (SEM) of LSPs (a: empty LSP; b: LSP with immobilized dried cells)(He et al., 2016)

Pentachlorophenol (PCP) is an organochlorine compound used as a pesticide and a disinfectant, and extremely toxic to humans from acute ingestion and inhalation exposure. It is shown that the sorption of PCP using nylon fiber with immobilized *R. oryzae* to eliminate the chemical compound is possible. *R. oryzae* was immobilized in nylon fiber cubes, demonstrating growth even in the presence of PCP, which inhibited growth. The fungus removed 88.6% and 92% of PCP in cultures with initial concentrations of 12.5 and 25 mg PCP L^{-1}, respectively, showcasing its potential for PCP removal when immobilized in nylon fiber. This study is the first to report the efficacy of *R. oryzae*, immobilized in nylon fiber for PCP removal (León-Santiestebán et al., 2011).

Azo dyes are the most common and adaptable type of synthetic dyes, and they are widely used in many areas, including food, tannery, printing, cosmetics, paper, textile dyeing, and color photography. Even though they are used extensively, azo dyes are acknowledged as a major class of environmental contaminants. Bagchi et al. (2021) showed the adsorption of the azo dye Reactive Blue 4 (RB4) using both dead and carboxy-methyl cellulose (CMC) immobilized *R. oryzae* as a biosorbent in batch scale. The experiments were carried out in a 250 mL Erlenmeyer flask with a dye concentration ranging from 200 to 500 mg/L in a 50 mL dye solution. Maximum RB4 adsorption capacity (97.44%) was observed under the following conditions: pH 3.0, temperature 30°C, initial dye concentration 200 mg/L, and contact time of 6 hours. Among the tested matrices, CMC was identified as the most effective carrier for immobilizing *R. oryzae* biomass.

4. Conclusion

In conclusion, the studies outlined a diverse range of immobilization techniques and support matrices employed for the cultivation of *Rhizopus* species, particularly *R. oryzae*, to enhance the production of various valuable compounds, including lipolytic enzymes, fumaric acid, biodiesel, LA and gallic acid. The challenges associated with conventional filamentous fungal fermentations, such as the aerobic nature requiring costly aeration and the complexity of controlling cell morphology, have been addressed through innovative immobilization strategies.

The investigation of immobilization methods for LA production with *R. oryzae* revealed the efficacy of methods like gel entrapment using Ca-alginate, polyurethane foam, and loofah sponge. These approaches demonstrated improved fermentation rates, LA transformation efficiency, and stability in repeated batch operations. Moreover, alternative matrices, such as honeycomb-inspired structures and polyvinyl alcohol-cryogel, exhibited enhanced mass transfer and LA production.

In enzyme production, immobilization of *R. oryzae* for lipase and polygalacturonase production using alginate beads, polyurethane foam, and tobacco industry wastewater highlighted the versatility of this fungus in biocatalysis.

For biodiesel production, *R. oryzae* cells which were immobilized on various matrices, including biomass support particles, reticulated polyurethane foam, and loofah sponge, showcased promising results. The studies highlighted the significance of support matrix selection for efficient transesterification of oils into fatty acid methyl esters.

R. oryzae cells immobilized in polyurethane foam, nylon fiber, and muslin fabric showed promise for environmental applications by efficiently eliminating pollutants such as PCP and RB4.

Overall, these studies contribute valuable insights into the application of immobilization techniques and support matrices to enhance the production of various compounds using *Rhizopus* species. The findings not only advance the understanding of fungal fermentation processes but also hold promise for industrial applications, offering sustainable and efficient methods for producing diverse bio-based products.

REFERENCES

Abd Razak, L. D., Abd Rashid, N. Y., Jamaluddin, A., Sharifudin, S. A., Abd Kahar, A., & Long, K. (2017). Cosmeceutical potentials and bioactive compounds of rice bran fermented with single and mix culture of Aspergillus oryzae and Rhizopus oryzae. *Journal of the Saudi Society of Agricultural Sciences*, *16*(2), 127–134.

Arumugam, A., & Ponnusami, V. (2014). Biodiesel production from Calophyllum inophyllum oil using lipase producing Rhizopus oryzae cells immobilized within reticulated foams. *Renewable Energy*, *64*, 276–282. https://doi.org/10.1016/j.renene.2013.11.016

Bagchi, M., Bera, D., & Adhikari, S. (2021). Biosorption of an azo dye Reactive Blue 4 from aqueous solution using dead and CMC immobilized biomass of Rhizopus oryzae (MTCC 262). *Bioremediation Journal*, *25*(4), 326–346. https://doi.org/10.1080/10889868.2021.1884526

Balla, E., Daniilidis, V., Karlioti, G., Kalamas, T., Stefanidou, M., Bikiaris, N. D., Vlachopoulos, A., Koumentakou, I., & Bikiaris, D. N. (2021). *Poly (lactic Acid): A Versatile Biobased Polymer for the Future with Multifunctional Properties — From Monomer Synthesis , Polymerization Techniques and Molecular Weight Increase to PLA Applications*.

Ban, K., Kaieda, M., Matsumoto, T., Kondo, A., & Fukuda, H. (2001). Whole cell biocatalyst for biodiesel fuel production utilizing Rhizopus oryzae cells immobilized within biomass support particles. *Biochemical Engineering Journal*, *8*(1), 39–43. https://doi.org/10.1016/S1369-703X(00)00133-9

Battaglia, E., Benoit, I., Brink, J. Van Den, Wiebenga, A., Coutinho, P. M., Henrissat, B., & Vries, R. P. De. (2011). Carbohydrate-active enzymes from the zygomycete fungus Rhizopus oryzae : a highly specialized approach to carbohydrate degradation depicted at genome level. *BMC Genomics*, *12*(1), 38. https://doi.org/10.1186/1471-2164-12-38

Cao, N., Du, J., Gong, C. S., & Tsao, G. T. (1996). Simultaneous production and recovery of fumaric acid from immobilized Rhizopus oryzae with a rotary biofilm contactor and an adsorption column. *Applied and Environmental Microbiology*, *62*(8), 2926–2931. https://doi.org/10.1128/aem.62.8.2926-2931.1996

Chiou, T. W., Wang, Y. C., & Liu, H. S. (1998). Utilizing the macroporous packed bed for insect cell/baculovirus expression. Part 2: The production of human interleukin-5 in polyurethane foam and cellulose foam packed bed bioreactors. *Bioprocess Engineering*, *18*(2), 91–100.

Das, R. K., Brar, S. K., & Verma, M. (2015). Enhanced fumaric acid production from brewery wastewater by immobilization technique. *Journal of Chemical Technology and Biotechnology*, *90*(8), 1473–1479.

Dotsenko, A. S., Dotsenko, G. S., Senko, O. V., Stepanov, N. A., Lyagin, I. V., Efremenko, E. N., Gusakov, A. V., Zorov, I. N., & Rubtsova, E. A. (2018). Complex effect of lignocellulosic biomass pretreatment with 1-butyl-3-methylimidazolium chloride ionic liquid on various aspects of ethanol and fumaric acid production by immobilized cells within SSF. *Bioresource Technology*, *250*, 429–438.

Efremenko, E., Spiricheva, O., Varfolomeyev, S., & Lozinsky, V. (2006). Rhizopus oryzae fungus cells producing L(+)-lactic acid: Kinetic and metabolic parameters of free and PVA-cryogel-entrapped mycelium. *Applied Microbiology and Biotechnology*, *72*(3), 480–485.

Elibol, M., & Özer, D. (2000). Lipase production by immobilised *Rhizopus arrhizus. Process Biochemistry*, *36*(3), 219–223.

Furusaki, S., & Seki, M. (1992). Use and engineering aspects of immobilized cells in biotechnology. *Adv Biochem Eng Biotechnol.*, *23*, 161–185.

Ganguly, R., Dwivedi, P., & Singh, R. P. (2007). Production of lactic acid with loofa sponge immobilized Rhizopus oryzae RBU2-10. *Bioresource Technology*, *98*(6), 1246–1251. https://doi.org/10.1016/j.biortech.2006.05.004

Ghosh, B., Karmakar, M., & Ray, R. R. (2015). Production of Extracellular Isoamylase and Endoglucanase by Entrapped Spores of Rhizopus oryzae PR7. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, *85*(3), 733–738.

Ghosh, B., & Ray, R. R. (2011). Current commercial perspective of Rhizopus oryzae: A review. *Journal of Applied Sciences*, *11*(14), 2470–2486. https://doi.org/10.3923/jas.2011.2470.2486

Gu, C., Zhou, Y., Liu, L., Tan, T., & Deng, L. (2013). Production of fumaric acid by immobilized Rhizopus arrhizus on net. *Bioresource Technology, 131*, 303–307. https://doi.org/10.1016/j.biortech.2012.12.148

Hamamci, H., & Ryu, D. D. Y. (1994). Production of L(+)-lactic acid using immobilized rhizopus oryzae reactor performance based on kinetic model and simulation. *Applied Biochemistry and Biotechnology*, *44*(2), 125–133. https://doi.org/10.1007/BF02921650

Hang, Y. D., Hamamcı, H., & Woodams, E. E. (1989). Production of L8+)-Lactic acid by Rhizopus oryzae immobilized in calcium alginate gels. *Biotechnology Letters*, *11*(2), 119–120.

He, Q., Shi, H., Gu, H., Naka, G., Ding, H., Li, X., Zhang, Y., Hu, B., & Wang, F. (2016). Immobilization of *Rhizopus oryzae* LY6 onto Loofah Sponge as a Whole-Cell Biocatalyst for Biodiesel Production. *BioResources*, *11*(1), 850–860.

Katzbauer, B., Narodoslawsky, M., & Moser, A. (1995). Classification system for immobilization techniques. *Bioprocess Engineering*, *12*(4), 173–179. https://doi.org/10.1007/BF01767463

León-Santiestebán, H., Meraz, M., Wrobel, K., & Tomasini, A. (2011). Pentachlorophenol sorption in nylon fiber and removal by immobilized Rhizopus oryzae ENHE. *Journal of Hazardous Materials*, *190*(1–3), 707–712. https://doi.org/10.1016/j.jhazmat.2011.03.101

López, E., Deive, F. J., & Longo, M. A. (2008). *Lipolytic Enzyme Production by Immobilized Rhizopus oryzae*. *11*, 1555–1560.

Lozinsky, V. I. (2008). Polymeric cryogels as a new family of macroporous and supermacroporous materials for biotechnological purposes. *Russian Chemical Bulletin*, *57*(5), 1015–1032.

Lusta, K. A., Chung, I. K., Sul, I. W., Park, H. S., & Shin, D. I. (2000). Immobilization of fungus Aspergillus sp. by a novel cryogel technique for production of extracellular hydrolytic enzymes. *Process Biochemistry*, *35*(10), 1177–1182. https://doi.org/10.1016/S0032-9592(00)00158-8

Maslova, O., Stepanov, N., Senko, O., & Efremenko, E. (2019). Production of various organic acids from different renewable sources by immobilized cells in the regimes of separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SFF). *Bioresource Technology*, *272*(September 2018), 1–9.

Misro, S. K., Kumar, M. R., Banerjee, R., & Bhattacharyya, B. C. (1997). Production of gallic acid by immobilization of Rhizopus oryzae.

Bioprocess Engineering, *16*(5), 257–260.

Mitchell, C. (1926). *Vinegar: its manufacture and examination* (2nd Editio). Griffin.

Naude, A., & Nicol, W. (2017). Fumaric acid fermentation with immobilised Rhizopus oryzae: Quantifying time-dependent variations in catabolic flux. *Process Biochemistry*, *56*, 8–20.

Nursiwi, A., Pertiwi, F., Ishartani, D., Sari, A. M., & Zaman, M. Z. (2021). Substrates and storage time evaluation for preparing tempeh starter from Rhizopus oryzae Substrates and storage time evaluation for preparing tempeh starter from Rhizopus oryzae CBS130145. *IOP Conference Series: Earth and Environmental Science*, *828*(012003), 1–6.

Özer Uyar, G. E., & Uyar, B. (2023). *Potato peel waste fermentation by Rhizopus oryzae to produce lactic acid and ethanol*. *April*, 5908–5917. https://doi.org/10.1002/fsn3.3670

Pimtong, V., Ounaeb, S., Thitiprasert, S., Tolieng, V., Sooksai, S., Boonsombat, R., Tanasupawat, S., Assabumrungrat, S., & Thongchul, N. (2017). Enhanced effectiveness of Rhizopus oryzae by immobilization in a static bed fermentor for L-lactic acid production. *Process Biochemistry*, *52*, 44–52. https://doi.org/10.1016/j.procbio.2016.09.020

Ranjit, C., & Srividya, S. (2016). Lactic acid production from free and Polyurethane immobilized cells of Rhizopus oryzae MTCC 8784 by direct hydrolysis of starch and agro-industrial waste. *International Food Research Journal*, *23*(6), 2646–2652.

Sebastian, J., Dominguez, K. V., Brar, S. K., & Rouissi, T. (2021). Fumaric acid production using alternate fermentation mode by immobilized Rhizopus oryzae-a greener production strategy. *Chemosphere*, *281*(November 2020), 130858. https://doi.org/10.1016/j.chemosphere.2021.130858

Shahri, S. Z., Vahabzadeh, F., & Mogharei, A. (2020). Lactic acid production by loofah-immobilized Rhizopus oryzae through one-step fermentation process using starch substrate. *Bioprocess and Biosystems Engineering*, *43*(2), 333–345. https://doi.org/10.1007/s00449-019-02231-5

Sizemore, S. R., Nichols, R., Tatum, R., Atanassov, P., Johnson, G. R., & Luckarift, H. R. (2013). Immobilization of Whole Cells by Chemical Vapor Deposition of Silica. In J. Guisan (Ed.), *Immobilization of*

Enzymes and Cells. Methods in Molecular Biology (3rd Editio, pp. 301–312). Humana Press, Totowa, NJ.

Sun, Y., Li, Y. L., Yang, H., Bai, S., & Hu, Z. D. (1996). Characteris-tics of immobilized *Rhizopus oryzae* in polyurethane foam cubes. *Biotechnology Techniques*, *10*(11), 809–814.

Tanyıldızı, M. Ş., Bulut, Ş., Selen, V., & Özer, D. (2012). Optimization of lactic acid production with immobilized Rhizopus oryzae. *African Journal of Biotechnology*, *11*(34), 8546–8552. https://doi.org/10.5897/ajb11.1706

Tay, A., & Yang, S. (2002). Production of L (+) -Lactic Acid From Glucose and Starch by Immobilized Cells of Rhizopus oryzae in a Rotating Fibrous Bed Bioreactor. *Biotechnology and Bioengineering*, *80*(1), 1–12. https://doi.org/10.1002/bit.10340

Tosa, T., & Shibatani, T. (1995). Industrial Application of Immobilized Biocatalysts in Japan. *Annals of the New York Academy of Sciences*, *750*(1), 364–375.

Trakarnpaiboon, S., Praneetrattananon, S., & Kitpreechavanich, V. (2018). Simultaneous saccharification and fermentation of L-(+)-lactic acid production from liquefied cassava starch by immobilized rhizopus oryzae in a 3 L airlift fermenter. *Chiang Mai Journal of Science*, *45*(1), 77–91.

Trelles, J. A., & Rivero, C. W. (2020). Whole Cell Entrapment Techniques. In J. Guisan, J. Bolivar, F. López-Gallego, & J. Rocha-Martín (Eds.), *Immobilization of Enzymes and Cells. Methods in Molecular Biology, vol 2100*. Humana, New York, NY. https://doi.org/https://doi.org/10.1007

Virkajärvi, I., & Linko, M. (1999). Immobilization: A revolution in traditional brewing. *Naturwissenschaften*, *86*(3), 112–122.

Wang, P., Chen, Z., Li, J., Wang, L., Gong, G., Zhao, G., Liu, H., & Zheng, Z. (2013). Immobilization of Rhizopus oryzae in a modified polyvinyl alcohol gel for L(+)-lactic acid production. *Annals of Microbiology*, *63*(3), 957–964. https://doi.org/10.1007/s13213-012-0549-x

Wang, Z., Wang, Y., Yang, S. T., Wang, R., & Ren, H. (2010). A novel honeycomb matrix for cell immobilization to enhance lactic acid production by Rhizopus oryzae. *Bioresource Technology, 101*(14), 5557–5564. https://doi.org/10.1016/j.biortech.2010.02.064

Yamane, T., & Tanaka, R. (2013). Highly accumulative production of l(+)-

lactate from glucose by crystallization fermentation with immobilized Rhizopus oryzae. *Journal of Bioscience and Bioengineering*, *115*(1), 90–95. https://doi.org/10.1016/j.jbiosc.2012.08.005

Yen, H. W., & Lee, Y. C. (2010). Production of lactic acid from raw sweet potato powders by rhizopus oryzae immobilized in sodium alginate capsules. *Applied Biochemistry and Biotechnology*, *162*(2), 607–615. https://doi.org/10.1007/s12010-009-8884-5

Zheng, Y., Wang, Y., Zhang, J., & Pan, J. (2016). Using tobacco waste extract in pre-culture medium to improve xylose utilization for l-lactic acid production from cellulosic waste by Rhizopus oryzae. *Bioresource Technology*, *218*, 344–350. https://doi.org/10.1016/j.biortech.2016.06.071

CHAPTER 8

ASSESSMENT OF RISK PROFILES OF MICROPLASTICS ON FISH

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ASSESSMENT OF RISK PROFILES OF MICROPLASTICS ON FISH

"Microparticles are anthropogenic deposits seen in marine and freshwater ecosystems, originating from fragmented parts of pollutants and breakdown of larger plastic uses (Cole et al., 2011). Microparticles are classified into two categories based on their formation. Industrial activities produce microparticles, known as primary microparticles (GESAMP, 2016). Primary microparticles include microbeads produced in cosmetics, microfibers used in textiles, and pellets from air blasting (Barnes et al., 2009). Secondary microparticles are produced by the photo-oxidative, thermal, or mechanical degradation of larger plastics (GESAMP, 2016). Secondary microparticles include plastic waste and discarded fishing nets (Boucher and Friot, 2020)."

1.Physicochemical Properties of Microplastics:

Microplastics possess several physicochemical characteristics, including:

- Hydrophobic surfaces that enable them to float.
- Transport properties that allow them to carry pollutants.
- The ability to absorb pollutants such as polychlorinated biphenyls.
- UV photo-oxidative degradation properties.
- Thermooxidative properties.
- Biodegradation and/or thermal degradation properties.
- Adhesiveness to biomass in biofilms (Hidalgo-Rulz et al., 2012; Wright et al., 2013).

2.Microplastic Polymers

Polyethylene (PE), polyvinyl chloride (PVC), polystyrene (PS), polypropylene (PP), and polyethylene terephthalate (PET) are common microplastics that represent approximately 90% of global plastic production (Venghaus, 2017).

3.Shapes of Microplastics

Microplastics are found in various shapes in environmental settings. Although they can be rectangular, tablet-shaped, spherical, cylindrical, or disk-shaped, they are predominantly observed in rounded or oval shapes (Esmeray

& Armutçu, 2020; Abu-Hilal & Al-Najjar, 2009). The shapes of microplastics vary depending on the duration they stay in the environment and the type of fragmentation process (Doyle et al., 2011).

Sources of Microplastics (Aslan, 2018; Cole et al., 2011; Yurtsever, 2015; Duis & Coors, 2016)

4.Primary Microplastic Sources

- Microbeads in personal care and cosmetic products, such as shampoos, soaps, toothpaste, eyeliner, mascara, lip gloss, sunscreen, deodorant, facial cleansing, and peeling gels, originating from consumers
- Special medical applications
- Drilling fluids for oil and gas exploration
- Industrial abrasives
- Pre-production plastics, scrap production, plastic recycling
- Raw materials, residues, and waste released into the environment during industrial processes such as plastic processing, shaping, and production

5.Secondary Microplastic Sources

- Microplastics originating from plastic materials used in kitchens and exteriors of homes due to wear and tear
- Disposal of general waste and plastic waste
- Losses during waste collection from landfills and recycling facilities
- Plastic material loss during natural disasters
- Plastic mulching
- Synthetic polymer particles used to improve soil quality and as compost additives
- Wear from synthetic textile materials produced from polymers such as polyamide (nylon), polyester, polar, and acrylic used in clothing
- Fiber release from hygiene products
- Microplastics from transportation (tire abrasion) and synthetic polymer-based paints
- Wear and tear from other plastic materials
- Plastic products in organic waste

- Material lost or discarded from fishing and commercial vessels and aquaculture facilities

6.Classification of Microplastics

To classify microplastics, several parameters are considered, including their sources, the type of materials they are made from, shape, structure, color, and degree of wear (Yurtseven, 2016) (Figure 1).

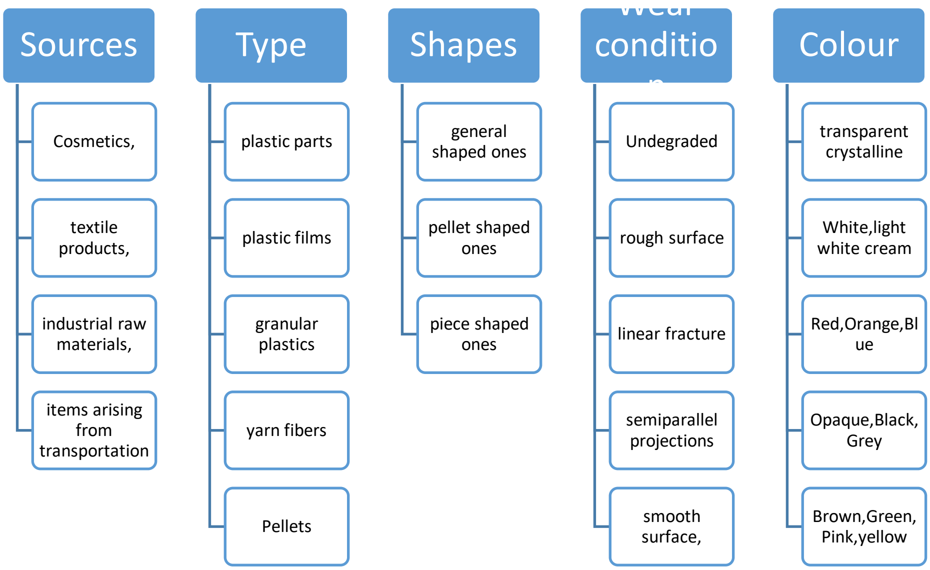

Figure 1: Classification of Microplastics (Yurtseven. 2016)

7.Toxic Effects of Microplastics

Plastic waste, due to its long lifespan and durability, can persist in the environment for many years without degrading. Moreover, microplastics have the ability to adsorb toxic organic chemicals such as antibiotics, organochlorine pesticides, endocrine disruptors, and heavy metals (Rochman et al., 2013). During the production of plastics, toxic substances such as heavy metals like lead, copper, cadmium, as well as materials with toxic effects such as phthalates and bisphenol A, are utilized. The smaller-sized microplastics generated by the breakdown of these plastics also contain the same heavy metals and toxic substances. Microplastics have the capability to absorb various organic and inorganic toxic substances present in waters, accumulating them on their surfaces. They can transport these absorbed toxic substances, which they hold on their surfaces, to organisms (Brennecke et al., 2016; Koelmans et al., 2016).

8.The Impact of Microplastics on Human Health

Microplastics can enter the human body through various routes, including oral consumption (drinking water, seafood, and other food items), dermal exposure, and inhalation, although the latter is considered less likely but possible, especially in the case of injured skin (Brate et al., 2016; Lehner & Weder, 2019).

Studies have observed the presence of microplastics in waters consumed by humans (tap and bottled water), daily food items (such as salt, fish, squid, mussels, shrimp, honey), and beverages like soda (Kosuth et al., 2018; Rochman et al., 2013; Yurtsever, 2016). A study conducted by Cox and colleagues in 2019 revealed that an average American adult and child, consuming as much as analyzed substances have needed, could be exposed to microplastics ranging from 81,000 to 123,000 particles annually (Cox et al., 2019).

In a study by Sharma and Chatterjee in 2017, it was suggested that the intake of microplastics could induce mutations in human chromosomes, potentially leading to infertility. Additionally, they reported that microplastics might be linked to obesity and even cancer (Cox et al., 2019).

Microplastics can negatively affect biological processes such as cell apoptosis, oxidative stress, and tissue necrosis. This raises concerns about their potential carcinogenic effects (Lu et al., 2015). A study by Schwabl and colleagues in 2018 examined stool samples from individuals in eight different countries (Austria, Finland, UK, Italy, Japan, Netherlands, Poland, and Russia) who included aquatic products in their routine diet. The study reported the presence of 9-24 microplastic particles sized between 50-500 μm in 10 grams of stool samples (Liebman et al., 2018).

It has been shown that interaction with plastic particles can induce immune responses and inflammation in cells. Microplastics, due to their small size, can interact with various organisms after translocation, leading to inflammation (Rochman et al., 2013; Yurtsever, 2016). Moreover, microplastics can accumulate on the skin, potentially causing dermal issues (Cox et al., 2019).

9.The Impact of Microplastics on Fish:

Fish, being a vital source of protein essential for human development, are susceptible to contamination by microplastics, posing a significant concern. When exposed to microplastics either independently or in conjunction with other pollutants, fish can experience various health issues, including:

- Oxidative Stress
- Internal Organ and Tissue Damage
- Changes in Immune-Related Gene Expression and Antioxidant Levels
- Accumulation, Blockage, and Damage in the Gastrointestinal System
- Release of False Fecal Pellets Disrupting Energy Transfer in Organisms

These effects can lead to physiological functions such as reduced swimming speed, internal blockages, decreased vitality, alterations in community composition of aquatic organisms, impaired digestion, inadequate nutrition and growth, organ system dysfunction, weakened immune systems, infertility, various respiratory and circulatory issues, and in severe cases, organ failure and death (James & Turner, 2020; Kumar et al., 2021; Triebskorn et al., 2019; Wang et al., 2020; Zhang et al., 2019).

Even when not ingested, microplastics can have a detrimental impact on fish and their behaviors. For instance, the attachment of microplastics to gills and skin can alter oxygen consumption and ion regulation, leading to respiratory stress (Abdel-Tawwab et al., 2019; Watts et al., 2016). Furthermore, impaired movement in fish can have a significant effect on predation and prey dynamics, affecting their survival (higher predation) or growth rates (feeding efficiency) and potentially leading to population declines (Little & Finger, 1990).

Additionally, evidence of microplastic ingestion has been reported in various species in both freshwater and marine systems, including plankton, copepods, zooplankton, crabs, small fish, turtles, fish larvae, seabirds, and mammals, with more than 150 fish species being affected (Jabeen et al., 2017).

10.Preventive Measures:

To address these issues, several measures can be taken:

Control the amounts of microplastics used in the production of products such as personal care items, toothpaste, exfoliating products, and detergents, and replace them with alternatives.

Limit the use of plastic in food packaging and encourage the use of glass and paper packaging.

Restrict the production and use of synthetic textile products.

Avoid unnecessary packaging and discourage the purchase of plastic-packaged products.

Establish an effective waste collection and recycling system in cases where plastic usage is essential.

Avoid the production of non-biodegradable plastic materials and instead encourage the production of biologically degradable plastic products and/or recycled products, utilizing eco-friendly bacteria and enzymes.

Restrict the sale of single-use plastic products and provide incentives to producers aiming to reduce waste production, such as tax reductions/exemptions.

Invest more in sustainable technologies that reduce plastic waste.

In this context, legal regulations by institutions and manufacturers to limit microplastic and plastic usage are essential.

REFERENCES

Koelmans, A. A., Nor, N. H. M., Hermsen, E., Kooi, M., Mintenig, S. M., & De France, J. (2019). Microplastics in freshwaters and drinking water: Critical review and assessment of data quality. Water research, 155, 410-422.

Abdel-Tawwab, M., Monier, M. N., Hoseinifar, S. H., & Faggio, C. (2019). Fish response to hypoxia stress: growth, physiological, and immunological biomarkers. Fish physiology and biochemistry, 45, 997-1013.

Abu-Hilal, A. H., & Al-Najjar, T. H. (2009). Plastic pellets on the beaches of the northern Gulf of Aqaba, Red Sea. Aquatic Ecosystem Health & Management, 12(4), 461-470.

Aslan, R. (2018). Mikroplastikler: hayatı kuşatan yeni tehlike. Göller Bölgesi Aylık Hakemli Ekonomi ve Kültür Dergisi, 6(66), 61-67.

Au, S. Y., Bruce, T. F., Bridges, W. C., & Klaine, S. J. (2015). Responses of Hyalella azteca to acute and chronic microplastic exposures. Environmental toxicology and chemistry, 34(11), 2564-2572.

Barnes, D. K., Galgani, F., Thompson, R. C., & Barlaz, M. (2009). Accumulation and fragmentation of plastic debris in global environments. Philosophical transactions of the royal society B: biological sciences, 364(1526), 1985-1998.

Bhuyan, M. S. (2022). Effects of microplastics on fish and in human health. Frontiers in Environmental Science, 10, 250.

Boucher, J., & Friot, D. (2020). Microplastiques primaires dans les océans.

Borrelle, S. B., Rochman, C. M., Liboiron, M., Bond, A. L., Lusher, A., Bradshaw, H., & Provencher, J. F. (2017). Why we need an international agreement on marine plastic pollution. Proceedings of the National Academy of Sciences, 114(38), 9994-9997.

Brennecke, D., Duarte, B., Paiva, F., Caçador, I., & Canning-Clode, J. (2016). Microplastics as vector for heavy metal contamination from the marine environment. Estuarine, Coastal and Shelf Science, 178, 189-195.

Cole, M., Lindeque, P., Halsband, C., & Galloway, T. S. (2011). Microplastics as contaminants in the marine environment: a review. Marine pollution bulletin, 62(12), 2588-2597.

Cox, K. D., Covernton, G. A., Davies, H. L., Dower, J. F., Juanes, F., & Dudas, S. E. (2019). Human consumption of microplastics. Environmental science & technology, 53(12), 7068-7074.

Doyle, M. J., Watson, W., Bowlin, N. M., & Sheavly, S. B. (2011). Plastic particles in coastal pelagic ecosystems of the Northeast Pacific ocean. Marine environmental research, 71(1), 41-52.

Duis, K., & Coors, A. (2016). Microplastics in the aquatic and terrestrial environment: sources (with a specific focus on personal care products), fate and effects. Environmental Sciences Europe, 28(1), 1-25.

Esmeray, E., & Armutcu, C. (2020). Mikroplastikler, çevre-insan sağlığı üzerine etkileri ve analiz yöntemleri. Düzce Üniversitesi Bilim ve Teknoloji Dergisi, 8(1), 839-868.

GESAMP (2016) Mikroplastiklerin deniz ortamındaki kaynakları, akıbeti ve etkileri: küresel bir değerlendirmenin ikinci bölümü. İçinde: Kershaw PJ,RochmanCM(editörler)IMO/FAO/UNESCO IOC/UNIDO/WMO/IAEA/UN/UNEP/UNDP Deniz çevre korumasının bilimsel yönlerine ilişkin Ortak Uzmanlar Grubu. Rep.Stud. GESAMP Sayı 93, s 220

He, D., Luo, Y., Lu, S., Liu, M., Song, Y., & Lei, L. (2018). Microplastics in soils: Analytical methods, pollution characteristics and ecological risks. TrAC Trends in Analytical Chemistry, 109, 163-172.

Liebmann, B., Köppel, S., Königshofer, P., Bucsics, T., Reiberger, T., & Schwabl, P. (2018, June). Assessment of microplastic concentrations in human stool: Final results of a prospective study. In Conference on nano and microplastics in technical and freshwater systems (pp. 28-31). Medical University of Vienna.

James, E., & Turner, A. (2020). Mobilisation of antimony from microplastics added to coastal sediment. Environmental Pollution, 264, 114696.

Hidalgo-Ruz, V., Gutow, L., Thompson, R. C., & Thiel, M. (2012). Microplastics in the marine environment: a review of the methods used for identification and quantification. Environmental science & technology, 46(6), 3060-3075

Huang, J. N., Wen, B., Xu, L., Ma, H. C., Li, X. X., Gao, J. Z., & Chen, Z. Z. (2022). Micro/nano-plastics cause neurobehavioral toxicity in discus fish (Symphysodon aequifasciatus): Insight from brain-gut-microbiota axis. Journal of Hazardous Materials, 421, 126830.

Hurley, R. R., & Nizzetto, L. (2018). Fate and occurrence of micro (nano) plastics in soils: Knowledge gaps and possible risks. Current Opinion in Environmental Science & Health, 1, 6-11.

Jabeen, K., Su, L., Li, J., Yang, D., Tong, C., Mu, J., & Shi, H. (2017). Microplastics and mesoplastics in fish from coastal and fresh waters of China. Environmental Pollution, 221, 141-149.

Jabeen, K., Li, B., Chen, Q., Su, L., Wu, C., Hollert, H., & Shi, H. (2018). Effects of virgin microplastics on goldfish (Carassius auratus). Chemosphere, 213, 323-332.

Kosuth, M., Mason, S. A., & Wattenberg, E. V. (2018). Anthropogenic contamination of tap water, beer, and sea salt. PloS one, 13(4), e0194970.

Kumar, R., Sharma, P., Manna, C., & Jain, M. (2021). Abundance, interaction, ingestion, ecological concerns, and mitigation policies of microplastic pollution in riverine ecosystem: A review. Science of The Total Environment, 782, 146695.

Lehner, R., Weder, C., Petri-Fink, A., & Rothen-Rutishauser, B. (2019). Emergence of nanoplastic in the environment and possible impact on human health. Environmental science & technology, 53(4), 1748-1765.

Little, E. E., & Finger, S. E. (1990). Swimming behavior as an indicator of sublethal toxicity in fish. Environmental Toxicology and Chemistry: An International Journal, 9(1), 13-19.

Lu, L., Luo, T., Zhao, Y., Cai, C., Fu, Z., & Jin, Y. (2019). Interaction between microplastics and microorganism as well as gut microbiota: A consideration on environmental animal and human health. Science of the Total Environment, 667, 94-100.

Lusher, A. L., Mchugh, M., & Thompson, R. C. (2013). Occurrence of microplastics in the gastrointestinal tract of pelagic and demersal fish from the English Channel. Marine pollution bulletin, 67(1-2), 94-99.

Mu, X., Qi, S., Liu, J., Yuan, L., Huang, Y., Xue, J., ... & Li, Y. (2022). Toxicity and behavioral response of zebrafish exposed to combined microplastic and bisphenol analogues. Environmental Chemistry Letters, 1-8.

Nelms, S. E., Galloway, T. S., Godley, B. J., Jarvis, D. S., & Lindeque, P. K. (2018). Investigating microplastic trophic transfer in marine top predators. Environmental pollution, 238, 999-1007.

Peda, C., Caccamo, L., Fossi, M. C., Gai, F., Andaloro, F., Genovese, L., ... & Maricchiolo, G. (2016). Intestinal alterations in European sea bass Dicentrarchus labrax (Linnaeus, 1758) exposed to microplastics: preliminary results. Environmental pollution, 212, 251-256.

Rillig, M. C., Ziersch, L., & Hempel, S. (2017). Microplastic transport in soil by earthworms. Scientific reports, 7(1), 1362.

Rochman, C. M., Hoh, E., Kurobe, T., & Teh, S. J. (2013). Ingested plastic transfers hazardous chemicals to fish and induces hepatic stress. Scientific reports, 3(1), 1-7.

Singh, B., & Sharma, N. (2008). Mechanistic implications of plastic degradation. Polymer degradation and stability, 93(3), 561-584.

Thiel, M., Luna-Jorquera, G., Álvarez-Varas, R., Gallardo, C., Hinojosa, I. A., Luna, N., ... & Zavalaga, C. (2018). Impacts of marine plastic pollution from continental coasts to subtropical gyres—fish, seabirds, and other vertebrates in the SE Pacific. Frontiers in Marine Science, 238.

Triebskorn, R., Braunbeck, T., Grummt, T., Hanslik, L., Huppertsberg, S., Jekel, M., ... & Köhler, H. R. (2019). Relevance of nano-and microplastics for freshwater ecosystems: a critical review. TrAC Trends in Analytical Chemistry, 110, 375-392.

Van Cauwenberghe, L., & Janssen, C. R. (2014). Microplastics in bivalves cultured for human consumption. Environmental pollution, 193, 65-70.

Venghaus, D., & Barjenbruch, M. (2017). Microplastics in urban water management. Technical Transactions, 114(1), 137-146.

Wang, J., Tan, Z., Peng, J., Qiu, Q., & Li, M. (2016). The behaviors of microplastics in the marine environment. Marine Environmental Research, 113, 7-17.

Yurtsever, M. (2015). Mikroplastikler'e genel bir bakiş. Dokuz Eylül Üniversitesi Mühendislik Fakültesi Fen ve Mühendislik Dergisi, 17(50), 68-83.

Yurtsever, M. (2018). Microplastic pollution threat in table salt that an abiotic sea product.

Wang, W., Ge, J., & Yu, X. (2020). Bioavailability and toxicity of microplastics to fish species: a review. Ecotoxicology and environmental safety, 189, 109913.

Watts, A. J., Urbina, M. A., Goodhead, R., Moger, J., Lewis, C., & Galloway, T. S. (2016). Effect of microplastic on the gills of the shore crab Carcinus maenas. *Environmental science & technology*, *50*(10), 5364-5369.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. Environmental pollution, 178, 483-492.

Wright, S. L., & Kelly, F. J. (2017). Plastic and human health: a micro issue?. Environmental science & technology, 51(12), 6634-6647.

Wright, S. L., Thompson, R. C., & Galloway, T. S. (2013). The physical impacts of microplastics on marine organisms: a review. Environmental pollution, 178, 483-492.

Zhang, Y., Gao, T., Kang, S., & Sillanpää, M. (2019). Importance of atmospheric transport for microplastics deposited in remote areas. Environmental Pollution, 254, 112953.

Zhao, Y., Qin, Z., Huang, Z., Bao, Z., Luo, T., & Jin, Y. (2021). Effects of polyethylene microplastics on the microbiome and metabolism in larval zebrafish. Environmental Pollution, 282, 117039.

Zhang, X., Xia, M., Zhao, J., Cao, Z., Zou, W., & Zhou, Q. (2022). Photoaging enhanced the adverse effects of polyamide microplastics on the growth, intestinal health, and lipid absorption in developing zebrafish. Environment International, 158, 106922

CHAPTER 9

GOAT AND SHEEP PRODUCTION IN MEDITERRANEAN AGRO-ECOSYSTEM

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Introduction

The Mediterranean region has a very rich and long history of trade across the Mediterranean Sea in many industries, particularly in animal production. The Mediterranean animal industries and ruminants, in particular, have some common traits such as the importance of pastoral systems, light and young carcass productions, numerous labeled and regional products, and the predominance of small versus large ruminants (de Rancourt and Mottet, 2008). Moreover, sheep and goats play a crucial role in the farming economy of the Mediterranean basin thanks to their ability to exploit marginal areas, as well as the limited labor and capital required for their growth. In very difficult and degraded areas or harsh climates, this breeding type is the only possible primary activity able to prevent land abandonment (Enne *et al.,* 2004). The sustainability of livestock farming systems about climate change, population dynamics, food security, and provision of agro-ecosystem services has become a central issue of public and scientific debate.

The Mediterranean Basin also known as the Mediterranean region or sometimes Mediterranean is the region of lands located at the intersection of two major landmasses, Eurasia and Africa. Around the Mediterranean Sea, it stretches c.3,800 km east to west from the tip of Portugal to the shores of Lebanon and c.1,000 km north to south from Italy to Morocco and Libya (https://en.wikipedia.org/wiki/Mediterranean_Basin; Sundseth, 2009; Efe *et al.*, 2008). The climate is characterized by hot-humid summers and humid-cool winters but it can also be notoriously capricious with sudden torrential downpours or bouts of high winds (eg. the Sirocco, the Mistral) occurring at various times of the year. These climatic conditions have a profound influence on the vegetation and wildlife of the region (Sundseth, 2009). Moreover, the Mediterranean Region offers an ever-changing landscape of high mountains, rocky shores, impenetrable scrub, semi-arid steppes, coastal wetlands, sandy beaches, and a myriad islands of various shapes and sizes dotted amidst the clear blue sea (https://en.wikipedia.org/wiki/Mediterranean_Basin). Although much of the hotspot was once covered by a dense cover of forests, the Basin has experienced intensive human development and impact on its ecosystems for at least 8,000 years, significantly longer than any other hotspot. The greatest impacts have been deforestation, habitat fragmentation, intensive grazing and fires, and infrastructure development, especially on the coast, which have distinctly altered the landscape. The agricultural lands, evergreen woodlands, and maquis habitats dominating the basin are the result of these disturbances over several millennia (Efe et *al.*, 2008).

1.Socio-economic importance of small ruminant production

Small ruminants are important for income generation and security, food supply, and development in rural Mediterranean areas especially in mountainous and marginal regions (Chentouf, *et al.*, 2014). Production of small ruminants (sheep and goats) has considerable significant economic, social, and environmental value in West Asia, North Africa, and South European regions (Spain, France, Italy, and Greece) (Aw-Hassan *et al.*, 2008; de Rancourt *et al.*, 2004). Sheep and goat production being frequently the only possible production in less favored areas is often fundamental to maintain social activities and to keep vegetation out of fire danger (de Rancourt *et al.*, 2004). The animals provide the owners with milk, meat, wool/hair, leather, and dung and, in addition, can be used as a source of cash, for transport, and as pack animals (Degen, 2007). In North African economics, the livestock sector plays an important role, especially sheep, goats, and cattle which account for 25-80 percent of the value of agriculture output in the region. Moreover, sheep and goats contribute significantly to the livelihoods, self-employment, and food security of the rural poor (Bengoumi and Ameziane El Hassani, 2014). In the mountainous Mediterranean part of Turkey, goat farming is the most important animal production for people who don’t have any other alternative for their subsistence. In addition, milk and meat products from goats are significant for the population (Darcan *et al.*, 2005).

2.Relation between small ruminant production and agro-ecosystem

Livestock have a substantial impact on the world’s water, land, and biodiversity resources and contribute significantly to climate change. In many situations, livestock are a major source of land-based pollution, emitting nutrients and organic matter, pathogens, and drug residues into rivers, lakes, and coastal seas. Animals and their wastes emit gases, some of which contribute to climate change, as do land-use changes caused by the demand for feed grains and grazing land. The severity of grazing in the European region causes the gradual disappearance of fauna, flora, and entire biotopes, pollution of surface and ground waters, increase in soil salinization, and soil erosion, and, ultimately, intense changes in the landscape. These problems are even more serious in the Mediterranean basin: several regions in the Iberian Peninsula are already so degraded as to be included in the world map of deserts (Enne *et al.*, 2004; Steinfeld *et al.*, 2006). In addition, water availability is becoming a serious constraint to the expansion of agriculture and to meet other growing human needs. Agriculture is the largest user of water, accounting for 70 percent of total freshwater use. (Steinfeld *et al.*, 2006).

The main features of sheep and goat production systems relate to the way small ruminants are raised. The major criteria adopted to distinguish the various systems are based on feed resources used and the nature of flock movement during the annual cycle (Nefzaoui *et al.*, 2011). Small ruminant systems of the Mediterranean basin have considerable economic, social, and environmental importance (Toro-Mujica, *et al.*, 2015). According to Boyazoglu (1989) reported by Bocquier *et al.* (2006) affirmed that in the Mediterranean area, the traditional dairy system classified from the land utilization implies three different breeding systems: sedentary, with transhumance and nomadic. Nefzaoui *et al.* (2011) concluded that in these areas there are also semi-sedentary systems, oasis systems, and peri-urban system.

a) Nomadic and Transhumant system: In the past, the nomadic and transhumant production systems were dominant. In summer and autumn, the flocks move to the cultivated areas to graze cereal stubble and the residues of irrigated crops. During the last decades, transhumance has been gradually abandoned. The majority of transhumant flocks were turned into sedentary flocks (Katanos *et al.*, 2005; Nefzaoui *et al.*, 2011). In the nomadic system, the flocks migrate annually from the lowland winter ranges to the higher mountain grazing areas in the summer. It is the common system used for small ruminants in the driest areas of North Africa and Near East (NENA) countries (Rajab-baig and Kamalzadeh, 2011; Nefzaoui *et al.*, 2011). Both nomadic and transhumant systems are characterized by low productivity and high grazing pressure in arid zones (Nardone, 2003). Sheep and goats raised by pastoralists are generally low-producing in terms of milk and offspring but are well-adapted to the climatic conditions and are relatively tolerant of local diseases (Degen, 2007).

b) Semi-sedentary system: In this system, the flocks are kept in cultivated areas during the winter season. However, in February they must leave the cultivated areas for grazing areas in the steppe to avoid crop damage. Animals are moved back into cultivated areas immediately after crop harvest to graze cereal stubbles. By the end of summer, the flocks are moved back to their villages (Nefzaoui *et al.*, 2011).

c)Sedentary system: In this system, sheep and goat flocks are kept at or close to the village or farm all year round. The sedentary herding system is dominant among the sheep and goat farming systems. During the day they are grazed either on the common village range or on privately owned or hired grazing areas; they also have access to stubbles and fallow fields. For the night they return to their sheds in the village or on the farm (Yalçin, 1986; Laga, *et al.,* 2005). These fenced systems of managed fodder areas or semi-natural grazing lands can be found in Turkey, Greece, Sardinia, Tuscany, Lazio, and Sicily, but especially in western Spain. Also, animals receive some hand-fed supplements in almost all seasons (Yalçin, 1986; Poux, 2006; Nefzaoui *et al.*, 2011).

d) Oasis system: The oasis system can allow the raising of improved goats thanks to the possibility of producing enough fodder and by-products to feed the animals (Djemali and Bedhiaf, 2005).

e) Peri-urban system: This system is more common in peri-urban areas, where hundreds of lambs are confined in a yard or barn for fattening. Traditionally barley is the main diet's ingredient (Nefzaoui *et al.,* 2011).

3. Factors limiting small ruminant production in the Mediterranean region

In the Mediterranean region, small ruminant populations are estimated 40,596,551 goats and 152,069,381 sheep in 2013 (FAOSTAT). These values account respectively for 4.04% and 12.97% of the world's population. Small ruminant farming systems are characterized by great diversity, in terms of types of production, breeds (more than 200 breeds), and levels of intensification, and are adapted to a wide range of situations. However, due to the climate conditions, dramatic feed deficits, shortage of drinking water, mismanagement and husbandry, poor control of animal health, weak interaction between researchers, farmers, extension workers, and policymakers, poor organization of herders, and unfavorable policies and legislations, the region could not reach its sufficiency on livestock products (Marie, 2011; Nefzaoui *et al.,* 2011; Bengoumi and Ameziane El Hassani, 2014).

3.1. Small ruminant production and climate change interactions

The Mediterranean is one of the most vulnerable European regions to climate change, e.g. in terms of future water shortages, losses of agricultural potential, and biome shifts (Hoff, 2013). Climatology characteristics such as ambient temperature and rainfall patterns have a great influence on pasture and food resources availability cycle throughout the year, and types of disease and parasite outbreaks among animal populations (Lamy *et al.,* 2012). Also, the ability of livestock to breed, grow, and lactate to their maximal genetic potential, and their capacity to maintain health is strongly affected by climatic features (Lacetera *et al.,* 2013). The performance, health, and well-being of livestock animals are strongly affected, directly or/and indirectly, by climate change and variability. These impacts are more severe in developing countries and also due to poor access to technologies and financial support (Ronchi and Nardone, 2006).

3.2. Impact of environmental stress on small ruminant reproduction

High ambient temperatures compromise the reproductive efficiency of farm female and male animals (Ben Salem et al., 2011). Environmental stresses affect the estrous behavior, embryo production, birth weights of lambs, placental size, and function, and fetal growth rate. It can lead to abnormal gametogenesis, folliculogenesis and ovulation, delayed onset of puberty, large anestrus periods, change in sexual behavior, increased embryo and pregnancy loss, low fertility and prolificacy, increased perinatal mortality and morbidity (Prasad *et al,* 2015; Sakly *et al.,* 2012; Sejian, 2012). In a study on the effect of climate changes on the estrus incidence of goats raised in the Mediterranean region of Turkey, Güney *et al.* (2009) reported that estrus onset has slipped to September which was occurring in August in the past due to the climate changes. During the period July to September, the estrus densities were reaching to the peak levels when the atmospheric temperatures were decreasing within years.

3.3. Impact of environmental stress on small ruminant milk production

Small ruminants (sheep and goats) are a major component of the dairy sector in the Mediterranean basin (Sotiraki *et al.*, 2013). The productive cycle of Mediterranean sheep and goats is very closely linked to the Mediterranean climate. Sheep and goat milk production in the Mediterranean basin is mostly based on pasture utilization and thus follows the pasture availability pattern. This causes a strong seasonal pattern to the amount of milk processed by cheese processing plants, with the peak being in the spring, a marked reduction in early summer, and low availability of milk from August to October to November (autumn) (Todaro *et al.*, 2015). Also, depression in feed intake and reduction of milk production are commonly observed in heat-stressed goats (Rosa *et al.*, 2013).

3.4. Impact of environmental stress on pasture availability

The indirect impacts of climate change on livestock are in reducing water and pasture availability and other feed resources (Sejian, *et al.*, 2015). Fodder resources provide the principal sources of feed throughout the humid and sub-humid, arid semi-arid, and Mediterranean regions. They include grasses and legumes, and fibrous crop residues (FCR) (Devendra, 1996). Additionally, in the Mediterranean basin, for instance, high temperatures and lack of rainfall limit pasture growth in summer. The grazing capacity of mountainous grassland declines significantly during autumn and thus, the animals have to be removed into lowlands where cultivated winter cereals are used for grazing. In general, there is a difficulty in ensuring annual regularity of food supply to small ruminants and this is one of the major constraints for the mainly extensive systems (Nardone *et al.*, 2004).

3.5. Impact of thermal stress on small ruminant feeding and growth

The best-recognized effect of raised body temperature is an adaptive depression of the metabolic rate associated with reduced appetite (Silanikove, 2000). These climatic changes impact animal feeding in two ways: quality and availability of feed-grain, pastures, forage crops and animals feed and water intake. Water, feed, and forage are the most important inputs for livestock production. Climate change affects livestock production by altering the quantity and quality of feed available to animals. It is also expected to change the species composition (and hence biodiversity and genetic resources) of grasslands and affect the digestibility and nutritional quality of forage. (Hoffmann, 2008; Thornton *et al.*, 2009).

According to Ames *et al.* (1980) reported by Lacetera *et al.* (2003) when growing animals suffer from heat stress average daily gain is lowered and the gross efficiency of converting nutrients to tissue is reduced. In a comparative study between cooled and not-cooled male Assaf lambs' growth subjected to heat stress, Darcan and Daskiran (2011) reported that at an air temperature averaged 35°C, the growth of male Assaf lambs was affected positively by fan treatment (1000-1500 hours during 9 weeks), live weight increased by 15%, and cooled lambs had higher thyroxin levels. Total feed intake of crossbred (75% Alpine and 25% Hair Goat) male kids was affected by ventilation and shower and their interaction effects ($P < 0.05$). More daily gain and final weight were affected by ventilation ($P < 0.05$) and ventilation x shower interaction ($P < 0.01$), as well. Feed intake increased with shower (58 vs. 63 kg/kid, $P < 0.05$) and ventilation application (58 vs. 62 kg/kid, $P < 0.05$), significantly (Darcan and Çankaya, 2007).

3.6. Impact of climate change on the development of small ruminant diseases

The most important environmental factors affecting the pathogens are temperature, humidity, and CO2 level (Mirski *et al.*, 2012). Biotic stress factors, in particular, gastrointestinal nematodes (helminths), which are very strongly influenced by both the short-term weather and climate through effects on their free-living larval stages on pasture and respiratory diseases are the major disease crises in small ruminants. Gastrointestinal parasites can cause production losses, increased susceptibility to other diseases and/or pests, and even death (Rahal, *et al.*, 2014; Sotiraki *et al.*, 2013; Van Dijk, 2009).

Furthermore, Sejian (2013) reported that changes in temperature and precipitation regimes may result in the spread of disease and parasites or produce a high incidence of diseases and mortality with concomitant decrease small ruminant's productivity. More seasonal rates of ovine parasitic gastroenteritis diagnosis suggest that, in line with temperature increases, fewer larvae of *Teladorsagia* and *Trichostrongylus* species survive the winter and spring at pasture, while the windows of transmission of these species, and *Haemonchus contortus*, have extended into the autumn. For all species categories significant differences in rates of diagnosis, and in the seasonality of disease, were identified between regions (Van Dijk, 2008).

3.7. Other factors

Other factors that negatively affect small ruminants and don't allow the region to reach its sufficiency on livestock products are mismanagement and husbandry, poor control of animal health, weak interaction between researchers, farmers, extension workers, and policymakers, poor organization of herders, and unfavorable policies and legislations, (Marie, 2011; Nefzaoui *et al.*, 2011; Bengoumi and Ameziane El Hassani, 2014).

4. Strategies to promote the sustainability of small ruminant production in the Mediterranean region

4.1. Utilization of biotechnology in small ruminants' production

Significant progress in research was achieved during recent years with small ruminants, particularly in the field of biotechnology. Indeed, the biotechnologies associated with reproduction (e.g. in vitro fertilization, in vitro embryo growth and transfer) are considered highly promising for the future of the small ruminant sector (Ronchi and Nardone, 2003; Morand-Fehr and Boyazoglu, 1999). In addition, the identification of genetic mechanisms explaining the polymorphism of certain characters, e.g. alpha-s1 casein content of goat milk can have interesting consequences in future selection programs. However, there are still large discrepancies between the availability of scientific knowledge and its application in sheep and goat husbandry in most production areas. Consequently, a strong effort has to be made to improve research efficiency, particularly technology transfer. Due to the high research costs, the organization of regional research and development networks should allow, in the end, the best results. (Morand-Fehr and Boyazoglu, 1999).

4.2. Feeding improvement strategies for sustainability of small production

Nutrition is one of the most important environmental factors contributing to unsatisfactory reproductive outputs. Under semi-extensive systems, additional feed (like secondary cereal grains) provided at critical physiological stages (usually corresponding to mating seasons or end of pregnancy and suckling stages); although not quantitatively important, can have a major impact on the productivity of the flocks when the animals are experiencing drought periods and their body condition is deteriorated. Decades of research on nutrition-reproduction interactions have led to the development of "focus feeding", the success of which depends on the timing and quality of the dietary stimulus as well as the metabolic history of the animals (particularly "metabolic memory"). Focus feeding is already being used to boost sperm production, increase ovulation rate, and improve offspring survival (Sakly *et al.*, 2012; Blache and Martin, 2009).

Sakly *et al.* (2012), carrying out a study on the reproductive response of Barbarine ewes to supplementation with alternative feed before and during mating under semi-arid extensive conditions in a semi-arid region with a Mediterranean-type climate (Tunisia) found indicated that short-term nutritional supply with cactus cladodes favors follicle development and preserved ovulation rate in sheep in comparison to other conventional sources of supplement. It also improved fertility when used to supplement animals over a longer period. They concluded that cactus cladodes may be considered a less expensive alternative to conventional concentrate supplements for increasing reproductive efficiency in semi-arid regions without the use of exogenous hormones.

In addition, Yener *et al.* (2003) reporting Anonymous (2000) affirmed that the key factors for improving milk production are providing adequate feeds, milk prices satisfactory for the farmers, and efficient supporting services. For the production of adequate, quality roughage, arable land should be utilized in the best way possible. Technical principles need to be applied for the sustainable use of rangelands. It is suggested that the ratio of concentrate price/producer milk price should be realized around 2/1 and that of sterilized milk/producer milk price also around 2/1.

4.3. Animal Health Issues

In small ruminants farming systems, chemoprophylaxis has been widely used as the main strategy to control parasitic diseases. Frequent use of suppressive or therapeutic anthelminthic drugs is no longer considered sustainable (Ronchi and Nardone, 2003). However, Methods of protection of animals from epizootic diseases need to be further improved. This includes the development of cheap and efficient methods of animal identification, registration and control of movements, new biological products and means of immunization as well as the development of production technologies, which would incorporate sanitary and preventive measures in daily operations. In the globalized world, the highest level of protection can be guaranteed only by the simultaneous and harmonized policies and practices in all parts of the world. However, more efficient regional cooperation in research and the implementation of measures could be a great step towards safeguarding and protecting the animal industry in the Mediterranean (Rosati, 2011).

Conclusion

Small ruminants have important economic, social, and environmental value for the autochthon population living in rural Mediterranean areas, especially in mountainous and marginal regions. However, goats and sheep contribute to the degradation of climate causing climate change associated with increasing ambient temperature. These climatic changes are one of the most important factors affecting small ruminants. Decade studies have enabled to development of strategies to reduce waste from animal husbandry activities and the negative impact of animal husbandry on the environment. To become more efficient, research using more specific methods directed to the reality of small ruminant production will be helpful.

REFERENCES

Aw-Hassan, A., F. Shomo and L. Iniguez. 2008. Small ruminant production: challenges and opportunities for poverty alleviation in West Asia and North Africa. *ICARDA, Aleppo, Syria. iv + 23 pp.*

Ben Salem H., Rekik M., Lassoued N. and Darghouth M. A., 2011. Global Warming and Livestock in Dry Areas: Expected Impacts, Adaptation and Mitigation, Climate Change: Socioeconomic Effects *ISBN: 978-953-307-411-5, InTech, Available from:*

Bengoumi M. and Ameziane El Hassani T. 2014. Evolution and efficacy of transfer of technologies in small ruminant production systems in North Africa. Technology creation and transfer in small ruminants: roles of research, development services and farmer associations,. *Int. Centre for Advanced Mediterranean Agronomic Studies, 2014, p 7*

Blache D., Martin G.B., 2009. Focus feeding to improve reproductive performance in male and female sheep and goats: how it works and strategies for using it. *In : Papachristou T.G. (ed.), Parissi Z.M. (ed.), Ben Salem H. (ed.), Moran d-Fe r P. (ed.). Nutritional and foraging ecology of sheep and goats. Zaragoza : CIHEAM / FAO / NAGREF, 2009 . p. 351 -364 (Options Méditerranéennes : Série A. Séminaires Méditerranéens; n° 85).*

Bocquier F., Moulin C.H. & P. Hassoun, 2006. Typicity of Mediterranean sheep products: improvement of nutrition and feeding. Animal products from the Mediterranean area. *EAAP publication No. 119, 2006*

Chentouf M., López-Francos A., Bengoumi M., Gabiña D., 2014. Technology creation and transfer in small ruminants: roles of research, development services and farmer associations. *Options Méditerranéennes, A, n° 108:7.*

Daramola J. O., Abioja M. O. and Onagbesan O. M., 2012. Heat Stress Impact on Livestock Production. Environmental Stress and Amelioration in Livestock Production, *Springer-Verlag Berlin Heidelberg 2012, pp 52-73*

Darcan N. K. and Daskiran I., 2011. Effects of ventilation of the sheep house on heat stress, growth and thyroid hormones of lambs. *Trop Anim Health Prod. 2011; 43(6):1123-7*

Darcan N. and Çankaya S., 2007. The effects of ventilation and showering on fattening performances and carcass traits of crossbred kids. *Small Ruminant Research (Impact Factor: 1.13). 03/2008; 75(2):192-198*

Darcan N. and Güney O., 2007. Alleviation of climatic stress of dairy goats in Mediterranean climate. *Small Ruminant Research, Volume 74, Issues 1-3, January 2008, Pages 212–215.*

Darcan N. Budak D. and Kantar M., 2005. Characterization of goat production in East Mediterranean region of Turkey. *Journal of Biological Science 5(6): 694-696.*

Degen A.A., 2007. Sheep and goat milk in pastoral societies. *Small Ruminant Research Volume 68, Issues 1-2, March 2007, pp 7–19*

Devendra C., 1996. Use of fodder resources by ruminants in warm climate countries. *Ann zootech (1996), 45, suppl 11-20. Elsevier/INRA*

de Rancourt M., Fois N., Lavín M.P., Tchakérian E., Vallerand F. 2004. Mediterranean sheep and goats production: An uncertain future. *Small ruminant research, Elsevier, Volume 62, Issue 3, Pages 167-179*

de Rancourt M., and Mottet A., 2008. Mediterranean animal production: Development or decline? *Options Méditerranennes, Series A, No. 78, Mediterranean seminars 2008, pp 13-22*

Djemali M. and Bedhiaf S., 2005. Genetic threats and potentials to improve native goats in Tunisia. Animal production and natural resources utilisation in the Mediterranean mountain areas. *EAAP publication No. 115, 2005*

Efe R., Cravins G., Ozturk M. and Atalay I. 2008. Natural Environment and Culture in the Mediterranean Region. *Cambridge Scholars Publishing 2009,*

Enne G., Zucca C., Montoldi A. and Noe L., 2004. The Role of Grazing in Agropastoral Systems in the Mediterranean Region and their Environmental Sustainability. *Advances in GeoEcology 37, CA TENA VERLAG. 35447 Reiskirchen, 2004.*

Gürsoy O. 2004. Economics and profitability of goat and sheep production under new support regimes and market conditions. Book of abstract of 55th Annual Meeting of the European Association for Animal Production. *Wageningen Academic Publisher the Netherlands 2004*

Hoff H., 2013. Vulnerability of Ecosystem Services in the Mediterranean Region to Climate Changes in Combination with Other Pressures. *Regional Assessment of Climate Change in the Mediterranean. Volume 2: Agriculture, Forests and Ecosystem Services and People. Springer Science+Business Media Dordrecht 2013*

Lacetera N., Bernabucci U., Ronchi B. and Nardone A., 2003. Physiological and productive consequences of heat stress. *The case of dairy ruminants. EAAP Technical series n°. 7*

Lacetera N., Segnalini M., Bernabucci U., Ronchi B., Vitali A., Tran A., Guis H., Caminade C., Calvete C., Morse A., Baylis M., and Nardone A. 2013. Climate Induced Effects on Livestock Population and Productivity in the Mediterranean Area. *Regional Assessment of Climate Change in the Mediterranean. Volume 2: Agriculture, Forests and Ecosystem Services and People. Springer Science+Business Media Dordrecht 2013*

Laga V., Katanos I., Skapetas B. and Chliounakis S., 2005. Transhumant sheep and goat breeding in Serres district of Central Macedonia, Greece. *Animal production and natural resources utilisation in the Mediterranean mountain areas. EAAP publication No. 115, 2005*

Lamy E., van Harten S., Sales-Baptista E., Manuela M. Guerra M. and Martinho de Almeida A., 2012. Factors Influencing Livestock Productivity. *Environmental Stress and Amelioration in Livestock Production. Springer-Verlag Berlin Heidelberg 2012*

Mavrogenis A. P. and Sinapis E., 2003. A review on goat production in east and South Mediterranean region Prospect for a sustainable of dairy sector in the Mediterranean. *EAAP N° 99 2003*

Mirski T., Bartoszcze M., Bielawska-Drózd A., 2012. Impact of Climate Change on Infectious Diseases. *Pol. J. Environ. Stud. Vol. 21, No. 3 (2012), 525-532*

Morand-Fehr P. and Boyazoglu J., 1999. Present state and future outlook of the small ruminant sector. *Small Ruminant Research, Elsevier, Volume 34, Issue 3, Pages 175–188*

Nardone A., Zervasb G., Ronchi B., 2004. Sustainability of small ruminant organic systems of production. *Livestock Production Science 90 (2004) 27–39*

Nardone A., 2003. Dairy farming systems: critical analysis and identification of the factors limiting their development in the Mediterranean area. *Prospects for a Sustainable Dairy Sector in the Mediterranean*. *EAAP Publication No. 99, 2003*

Prasad C.S., Malik P.K. and Bhatta R., 2015. Overview. *CAB International 2015 pp 1-7*

Poux X., Beaufoy G., Bignal E., Hadjigeorgiou I., Ramain B., Susmel P., 2006. Study on environmental consequences of Sheep and Goat farming and of the Sheep and Goat premium system. *Final report, European Commission/Directorate-General for Agriculture and Rural Development, July 2006*

Rahal A., Ahmad A. H., A. Prakash, R. Mandil, and A. T. Kumar., 2014. Environmental Attributes to Respiratory Diseases of Small Ruminants. *Veterinary Medicine International Volume 2014 (2014), Article ID 853627, 10 pages*

Rajab-baigy M. and Kamalzadeh A., 2011. The scope of livestock production in developing countries: a case study of Iran. *International Business: Research, Teaching and Practice 2011 5(1)*

Ronchi B. and Nardone A., 2006. Effects of climate variability on animal productions. Animal products from the Mediterranean area. *Wageningen Academic The-Netherlands, 2006*

Ronchi B. and Nardone A., 2003. Contribution of organic farming to increase sustainability of Mediterranean small ruminants livestock systems. *Livestock Production Science 80 (2003) 17–31*

Rosati A., 2011. Broad-view research in the Mediterranean system. New trends for innovation in the Mediterranean animal production. *Wageningen Academic Publishers the Netherlands, 2011*

Rosa A., Palucci A. , Zumbo A., 2013. Climatic effect on milk production of Camosciata goats reared in Calabria. *Journal Large Animal Review 2013 Vol. 19 No. 2 pp. 73-78*

Sakly C., Rekik M., Ben Salem I., Lassoued N., Mtaallah B., Kraïem K., Gonzalez-Bulnes A., 2012. Reproductive response of Barbarine ewes to supplementation with alternative feed prior to and during mating under semi-arid extensive conditions. *Animal farming and environmental* *interactions in the Mediterranean region, Wageningen Academic Publishers, the Netherlands, 2012*

Sejian V., Bhatta R., Soren N. M., Malik P. K., Ravindra J. P., Cadaba S. Prasad , and Lal R., 2015. Introduction to concept of climate change impact on livestock and its adaptation and mitigation. *Climate Change Impact on Livestock: Adaptation and Mitigation, Springer India 2015, pp1-25*

Sejian V., 2013 Climate change: Impact on production and reproduction, adaptation mechanisms and mitigation strategies in small ruminants: A review. *Indian Journal of Small Ruminants (The), 2013, Volume: 19, Issue: 1.*

Sejian V., 2012. Introduction. *Environmental Stress and Amelioration in Livestock Production, Springer-Verlag Berlin Heidelberg 2012, pp 2-18*

Steinfeld H., Gerber P., Wassenaar T., Castel V., Rosales M., de Haan C., 2006. *Livestock's long shadow-environmental issues and options, FAO 2006.*

Silanikove N., 2000. Effects of heat stress on the welfare of extensively managed domestic ruminants. *Livestock Production Science, Volume 67, Issues 1-2, Dec. 2000, pp 1-18.*

Sotiraki S., Stefanakis A., Hoste H., Maurer V., Butler G., Leifert C., 2013. The role of biotic and abiotic stress factors on sheep welfare: The example of parasites and climatic changes in European countries. *Options Méditerranéennes, A no. 107, Mediterranean seminars 2013.*

Sundseth K., 2009. Natura 2000 in the Mediterranean Region. *European Communities, 2009 pp 3*

Todaroa M., Dattenab M., Acciaiolic A., Bonannoa A., Brunid G., Caropresee M., Melef M., Sevie A., Trabalza Marinuccig M., 2015. Aseasonal sheep and goat milk production in the Mediterranean area: Physiological and technical insights. *Small Ruminant Research xxx (2015) xxx–xxx*

Van Dijk J., David G.P., Baird G., Morgan E.R., 2008. Back to the future: Developing hypotheses on the effects of climate change on ovine parasitic gastroenteritis from historical data. *Elsevier, Veterinary Parasitology, Volume 158, Issues 1–2, 25 November 2008, pp 73–84.*

Van Dijk J., Sargison N. D., Kenyon F. and Skuce P. J., 2009. Climate change and infectious disease: helminthological challenges to farmed ruminants in temperate regions. *Animal/ Volume 4 / Issue 03 / March 2010, pp 377-392.*

Yalçin B. C., 1986. Sheep and goats in Turkey. FAO Animal Production and Protection Paper 60. *Food and Agriculture Organization of the United Nations, Rome, 1986*

Yener S. M., Mansour H. and Cedden F., 2003. Mixed livestock production. *EAAP Publication No. 99, 2003.*

CHAPTER 10

NEW GENERATION FERTILIZER: ORGANOMINERAL FERTILIZERS

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INTRODUCTION

Modern agriculture, which has to renew itself, and scientific studies on these innovations are in a process of continuous change. The main purpose of continuous research of these innovations and change processes in agriculture is to obtain the highest efficiency from unit area in order to meet the rapid population growth and the ever-increasing food needs of people in parallel. It is known that it is necessary to use modern agricultural methods consciously and effectively to reach the desired high levels of productivity.

In order to increase the yield and quality in the production of plants, the main conditions are to provide the plant nutrients that the plants need and to maintain the fertility of the soil where the plants are grown. But, the needs of the nutrient of plants and plant varieties differ from each other. Therefore; nutritional management of plants, namely fertilization and at the same time ensuring the sustainability of soil fertility, has never lost its dynamism as long as human beings exist, and scientific research carried out depending on changing conditions has always been important.

The main purpose of fertilization, which is the main process for the nutritional management of plants, is to meet the nutritional needs of the plant throughout growth process and not to harm the environment and human factors while doing this. Due to all these reasons, the plant can benefit from fertilizer as a result of the fertilization process; It is directly related to soil, plant and climate effects, as well as plant nutrition management method and fertilizer use efficiency. Therefore; When fertilization, the suitability of the fertilizer to be applied into the soil should be checked for the plant variety, and the results of the product and soil analysis to be taken should be thoroughly evaluated. After the analysis results are evaluated, the appropriate amount of fertilizer dose should be calculated for the fertilizer to be applied, and the most appropriate fertilizer form according to soil and climate factors should be applied to the plant to be grown at the most appropriate time, with the most appropriate method and taking into account economic factors (Karaman, 2012). However, chemical fertilizer applications are made only to increase productivity without soil analysis; Although the use of chemical fertilizers has increased rapidly both in the world and in Turkey, a quality and sustainable management method has not been achieved in terms of efficiency in the use of chemical fertilizers (Karaman, 2012).

Incorrect and excessive chemical fertilizer applications to increase productivity; It causes the physical and chemical properties of the soil to change, the soil pH to go beyond the plant's requests, and the formation of ionic toxicity to the plants by creating a salt effect. In addition, incorrect and excessive fertilization can cause a decrease in soil biological diversity, a decrease in the resistance of plants to diseases and pests, a deterioration in the nutritional balance of products, pollution of water, and accumulation of heavy metals in the soil as a result of heavy metals in their composition mixing with the soil. In particular, these practices cause the organic matter levels of soils to decrease (Yıldız, 2018). As mentioned above, long-term incorrect and excessive use of chemical fertilizers negatively affects soils and can also cause various environmental problems due to groundwater pollution (Shan et al., 2015). Studies carried out to eliminate these negative effects; It shows that organomineral fertilizers can be used as an alternative to chemical fertilizers due to their effect on improving soil properties as well as their potential to provide plant nutrients (Erdal, 2018; Anonymous, 2018).

Organomineral fertilizers are considered as a class of fertilizers that are different from organic and chemical fertilizers in terms of their main properties, which are created by adding chemical fertilizers to organic wastes that improve soil properties and have fertilizer value. Organomineral fertilizers, called new generation fertilizers, are defined as solid and liquid products obtained by reacting or mixing one or more organic fertilizers alone, with compound, secondary or micro plant nutrient-containing chemical fertilizers (Kacar & Katkat, 2021). Organomineral fertilizers appears as a fertilizer that combine the soil-improving properties of organic matter and the benefits of chemical fertilizers in a single fertilizer.

The aim of this review is to give information about why organomineral fertilizers emerged and their benefits, and to try to convey the results of the studies on organomineral fertilizers carried out in the world and in our country.

1. PRODUCTION, CONSUMPTION, IMPORT AND EXPORT STATUS OF ORGANOMINERAL FERTILIZERS

While the fertilizer industry dates back to 1945-1950, research on the use of fertilizer for plant nutrition has been increasing in recent years. Especially the importance of the use of organomineral fertilizers is increasing day by day.

When the 2014 data of the Ministry of Agriculture and Forestry General Directorate of Plant Production (BÜGEM) are examined, it is seen that 306 companies received organic fertilizer production, marketing and import permits between 2003 and 2011. When Table 1 is examined, it is seen that the production of organomineral fertilizers in Turkey decreased from 2007 to 2012, and then increased in the following period. When examined in terms of consumption, it is seen that there is an increasing increase in the use of organic fertilizers every year, while there was no significant change in the use of organomineral fertilizers until 2012, and then it gradually increased in the following years and reached the maximum level in 2014. (Tunç, 2017). It is estimated that around 450 thousand tons of organomineral fertilizers were used in our country in 2021. In general, it is seen that the share of organomineral fertilizer use in the fertilizer sector is low. In order to increase this share, there is a need to change the usage habits of farmers and increase the use of organomineral fertilizers over time. For this purpose, the Ministry of Agriculture and Forestry has started to create a special support model for organomineral fertilizer support (Anonymous, 2021).

Table 1. Production and Consumption of Organic and Organomineral Fertilizers in Turkey by years (tons) (Tunç, 2017)

Years	Production		Consumption	
	Organic Fertilizers	Organomineral Fertilizers	Organic Fertilizers	Organomineral Fertilizers
2006	6,348.70	772.60	5.224.20	925.60
2007	9.8200.30	1.098.30	9.979.10	1,169.30
2008	13.958.50	284.70	13.945.50	433.30
2009	8.234.90	63.40	8.320.30	263.00
2010	11.493.10	58.50	11.591.80	250.00
2011	14.234.30	43.30	10.694.20	738.50
2012	10.253.90	53.10	8.590.90	1.436.70
2013	42.056.30	1.661.40	75.320.60	2.066.60
2014	170.716.10	69.097.50	190.879.20	126.741.00

When the import and export amounts of organic and organomineral fertilizers are examined; It is seen that the import and export of organomineral fertilizers increased towards 2014 (Table 2) (Tunç 2017).

Table 2. Import and export status of organic and organomineral fertilizers in Turkey by years (tons) (Tunç, 2017)

Years	Imports		Export	
	Organic Fertilizers	Organomineral Fertilizers	Organic Fertilizers	Organomineral Fertilizers
2006	293.40	206.60	0	0
2007	21.60	622.10	0.15	1.40
2008	123.30	297.10	0.30	2.30
2009	107.80	99.60	0	0.50
2010	129.30	133.70	1.60	0
2011	209.50	148.30	0.30	0
2012	77.80	170.30	15.00	0
2013	10,802.80	176.00	476.80	4.10
2014	31,386.80	91,365.60	66.20	45.40

Data after 2014 cannot be accessed because there is no separate classification according to the production, consumption, the import and export amount of organic fertilizers or organomineral fertilizers in the plant nutrition and industry statistics section of the Turkish Statistical Institute (TUIK) (Anonymous, 2021).

2. THE DEVELOPMENT, BENEFITS AND TYPES OF ORGANOMINERAL FERTILIZERS

The most important reason for the emergence of organomineral fertilizers is to combine the physical, chemical and biological benefits of organic matter in soils with the benefits of chemical fertilizers on plant production. With these properties, organomineral fertilizers also help to maintain these conditions by making soils suitable for plant production. In addition, organomineral fertilizers have a slow-release feature as they resist being washed away by precipitation and irrigation water. This feature of organomineral fertilizers is important in agricultural production and increases the benefit and effectiveness of fertilization (Kominko et al., 2016).

Soil, the world's thinnest layer, is the basis of terrestrial life. The natural structure of the soil consists of solid, liquid and gaseous substances. The proportions of these substances in the soil mass vary depending on the shape of the land, geological structure, climatic conditions and seasons. A loamy soil suitable for plant growth theoretically consists of 50% solids, 25% air and 25% water. Of the soil part, which constitutes 50%, 45% is composed of mineral substances and 5% of organic matter. As can be seen, although soil organic matter constitutes a very low part of the soil, it is the part that affects the soil ecosystem the most.

Soil organic matter includes microscopic organisms of soil microbial life as well as humus. Soil organic matter binds the sand, silt and clay particles in the soil, thus forming aggregates and improving the soil structure. As a result, it increases the resistance of the soil to erosion, reduces the formation of a layer of cream in the soil, allows more infiltration of rainwater into the soil (infiltration), regulates the movement of water and air in the soil by reducing soil compaction, increases soil temperature, water holding capacity of the soil and water uptake of plants, It reduces the loss of nutrients from the soil, increases the cation exchange and buffering capacity of the soil and enables plants to use more nutrients, reduces salt damage in crop production by increasing the buffering property against the change of soil reaction and soil salinization. In summary, soil organic matter is an important element of soil.

The level of organic matter, which is of great importance for soil fertility and sustainable agriculture, is very low in in almost all of the agricultural soils of our country. In order for the physical, chemical and biological properties and yield potential of agricultural soils to be at the desired levels, the organic matter content should be at least 3%. However, only 1% of Turkey's agricultural soil are above this level in terms of organic matter content. Organic and organomineral fertilizers have an extremely important role in order to close this the low organic matter deficit of our agricultural soils.

Organic fertilizers are fertilizers composed of plant, animal and human residues or wastes. The most important organic fertilizers are farm manure and animal waste. Organic fertilizers are fertilizers that contribute to the transformation of plant nutrients in the soil into plant-available form as well as improving the physical properties of the soil, which is important for plant growth (Tisdale and Nelson 1982). However, since it takes time for these fertilizers to break down and decompose in the soil, it also takes time for the plant to absorb the nutrients needed for the plant that continues its life cycle. In addition, the amounts of plant nutrients in organic fertilizers may vary, as well as the breakdown and decomposition of organic fertilizers. For this reason, these issues should be taken into consideration when using organic fertilizer. (Anonymous 2017a; Yıldız 2018).

Organomineral fertilizers, on the other hand, are types of fertilizers that are specifically formed as a result of the combination or reaction of single, double or triple plant nutrients with one or more organic products. These fertilizers contain both plant nutrients and at least 15% organic matter. Among organic fertilizers, chicken manure is one of the most important sources used in organomineral fertilizer production due to its high nitrogen content (Preusch et al., 2002). However, some natural fertilizers such as algae oil or fish oil are not preferred unless they are not necessary because they smell.

Organomineral fertilizers help plant roots to develop healthier by both increasing the aeration capacity of soils and providing carbon dioxide and water balance. In addition, they contribute to the increase in the development of stem, leaf and shoot development of plants due to the increase in the quality of soils. Organomineral fertilizers contain organic matter, humic and fulvic acid, nitrogen, phosphorus, potassium, sulfur, zinc and other micronutrients together and can be applied as base fertilizers. Organomineral fertilizers, like organic fertilizers, increase the water holding capacity of soils and contribute to making soils more fertile. In addition to all the benefits listed, the use of organomineral fertilizers will increase the use of organic fertilizers, which is low in our country. With the regulation prepared by the Ministry of Agriculture and Rural Affairs and published in the official gazette dated May 4, 2004 (Regulation on the Production, Import, Export, Market Supply and Supervision of Organic, Organomineral, Special Microbial and Enzyme Organic Fertilizers and Soil Regulators Used in Agriculture), organomineral fertilizers that can obtain appropriate production permits have become much more easily and reliably reachable (Anonymous, 2017b, Özkan 2013).

Organomineral fertilizers have been subjected to different classifications in terms of their production.

Nitrogenous organomineral fertilizers; These are fertilizers that contain organic nitrogen obtained from organic materials and nitrogen from one or more chemical fertilizers and the amount, form and solubility of the nitrogen contained must be clearly reported. They may contain other plant nutrients as secondary elements. However, the amount of phosphorus (P_2O_5) and potassium (K_2O) should not exceed 10% and 5%, respectively (Anonymous, 2003).

Nitrogen-phosphorus (NP) organomineral fertilizers; These are fertilizers that must contain organic nitrogen and phosphorus obtained from organic materials and their amounts must be clearly reported. In addition, the amount, form and solubility of nitrogen and phosphorus from nitrogen fertilizers, phosphorus fertilizers or single-nutrient chemical fertilizers must be reported. They may contain other plant nutrients and microelements. However, the reported amount of potassium (K_2O) should not exceed 5% (Anonymous, 2003).

Nitrogen-potassium (NK) organomineral fertilizers; They must contain organic nitrogen obtained from organic materials and their amounts must be clearly declared. In addition, the amount, form and solubility of potassium and nitrogen obtained from nitrogen-potassium (NK) fertilizers or simple chemical fertilizers must also be declared. They may contain other plant nutrients and microelements. However, the amount of declarable phosphorus (P_2O_5) should not exceed 10%. (Anonymous, 2003).

Nitrogen Phosphorus Potassium (NPK) organomineral fertilizers; They must contain nitrogen, phosphorus and potassium obtained from organic materials and their amounts must be clearly declared. In addition, the amounts, forms and solubility of nitrogen, phosphorus and potassium from compound chemical fertilizers or single chemical fertilizers must be declared. They may contain other plant nutrients and microelements. (Anonymous 2003).

3. SOME STUDIES CARRIED OUT WITH ORGANO-MINERAL FERTILIZERS IN TURKIYE

In the greenhouse experiment, Erdal et al. (2000) stated that the application of humic acid to soils in addition to phosphorus fertilizers in soils with problems in terms of available P increased the availability of phosphorus, increased the yield and quality of corn plant.

Yazıcı (2001) stated that in soils where B toxicity or Zn deficiency is a problem, deficiencies in plant growth and yield caused by these problems can be prevented by leonardite application.

Özcan et al. (2004) examined the effects of organic (farm manure and green manure) fertilizers and mineral nitrogen fertilization applied in different doses and mixtures on the protein and amino acid amounts of bread wheat varieties. In the study, they stated that the total protein amount of the grain increased as the nitrogen content increased, and the highest increase rate among the applications was obtained from the highest dose of mineral nitrogen fertilization supplemented by farmyard manure.

In their study conducted in Adana, Iğdırlı (2006) compared the effects of some organic and conventional practices on strawberry seedling yield and quality parameters. As a result of the research, they determined that all organic fertilizer applications during the experiment caused increases in seedling yield and quality.

Akıncı et al. (2007) investigated the effects of organomineral fertilizers and inorganic fertilizers on yield and yield components of bread wheat in their studies conducted in Diyarbakır in 2004-2005 and 2005-2006. As a result of the study, it was determined that yield, 1000 grain weight, grain hardness and plant height differed depending on fertilizer applications, while there was no difference in hectoliter weight and protein ratio. It was concluded that organomineral fertilizers were not as effective as inorganic fertilizers on the studied traits.

Karaca et al. (2005) applied chemical fertilizers containing leonardite, 6% and 9% NP separately and in combination to soils and investigated their contribution to biological properties and heavy metal contents of soils. According to the results of the study, the application of 6% NP+leonardite (as organomineral fertilizer) had the highest effect on biomass carbon, respiration and enzyme activity of soils. In addition, the Cd, Pb, Zn and Ni contents of the soils increased during the six-month incubation experiment when mineral fertilizer containing NP alone was applied to the soils, while a decrease in the amounts of these metals was observed when NP was applied in mixture with leonardite. Based on these results, researchers stated that leonardite has the ability to retain heavy metals contaminated with commercial fertilizer applications and that it provides positive benefits regarding the biological properties of the soil as well as soil pollution.

Pekcan et al. (2008) compared the effects of organomineral fertilizer, mineral and farmyard manure on the yield and quality of olives in their research. As a result of the five-year study, they stated that the highest yield per tree was obtained from organomineral fertilizer application.

Özalp (2010) investigated the effects of conventional fertilization and different organic fertilizer materials (chicken, pigeon, sheep, sewage sludge and commercial organic fertilizer) on yield and yield components of wheat plants. As a result of the study, the highest grain yield was obtained from pigeon manure application and the lowest grain yield was obtained from sheep manure application.

Zengin et al. (2010) examined the effects of mineral fertilizers and humic acid applications on yield and yield components in matador spinach. In field trials, ammonium sulfate (7 and 14 kg/da N) and DAP (4 and 8 kg/da P_2O_5) applied in two doses, liquid humic acid and humic acid+micro element was applied in increasing doses (0, 500, 1000 and 2000 ml/da). In the experiment, the effects of humic acid applications together with the use of mineral fertilizer were investigated. They stated that the effect of the applications on yield, average plant weight, leaf length and nutrient element content of the leaf were statistically significant, and the highest yield was obtained in the application of 2000 ml/da humic acid application with the use of mineral fertilizer.

Çalışkan & Ayan (2011) investigated the differences of some characteristics such as yield, plant height, stem height, stem width, number of branches, number of branches, wet and dry weight of nettle plants in Bafra region with different doses (0, 10, 15 and 20 kg/da) of organomineral fertilizers compared to NPK applications. they obtained the best growth in 20 kg/da organomineral fertilizer application and emphasized that increasing the amount of organomineral fertilizer increases the number of new branches up to a certain level.

Karaman et al. (2012); stated that that when the results of previous studies were examined, it was generally stated that the findings on the effect of organic fertilizers on Fe and Zn uptake differed, but were compatible with Mn uptake. According to the results of the research, they found that there were significant differences between the addition of humic acid to the soil and the availability of micronutrients and their uptake of micronutrients by plants.

Saglam et al. (2012) investigated the effects of leonardite and mineral nitrogen fertilizer application, separately or together, on the yield-yield components and plant nutrient contents of maize plant and found that the applications had positive effects. It was determined that the application of mineral nitrogen fertilizer with a dose of 200 kg/da of leonardite caused an increase in yield-yield components and nitrogen content of the plant. In the study, it was stated that the application of organic materials such as leonardite in agricultural areas should be supported with mineral fertilizers instead of being applied as a single input and that it is a necessity to adjust the doses well in this dual combination.

Demirtaş et al. (2012) investigated the effects of organic fertilizers, mineral fertilizers and their mixtures in different ratios on yield and quality in tomato cultivation under greenhouse conditions, and determined that mineral fertilizer + organic fertilizer applications gave the best results.

Özkan et al. (2012) investigated the effects of different ratios of organic fertilizer and mineral fertilizer, as well as soil mineral fertilizer and foliar organic fertilizer applications, on the nutritional status of the plant growth in greenhouse pepper cultivation. They determined that the N, P, K, Ca , Mg, Fe and Mn contents of leaf samples increased as a result of the applications. They also determined that organic and mineral fertilizer applications affected plant growth by increasing the height of the plants, the diameter of the main stem and side branches. As a result of the research, it was determined that more positive results were obtained when organic fertilizers were applied together with mineral fertilizers.

Özkan (2013) applied 0, 2.5, 5 g/m^2 nitrogen fertilizer doses and 4 g/m2 organomineral fertilizer to Bizet-1, Kokomo and Esquire English Grass (Loliumprenne L.) varieties. As a result of the research, it was determined that organomineral fertilizer application increased the dry matter amounts of English grass varieties. However, it was determined that the application of nitrogen fertilizers used in different doses together with organomineral fertilizer resulted in better growth in Kokomo variety compared to other varieties.

Günay (2014) applied different doses of mineral fertilizers and organomineral fertilizers to oil sunflower plants in Aydın Söke. In the study, it was determined that different fertilizers positively affected the nutrient contents of the plant and the use of organomineral fertilizers provided higher yield and quality in sunflower plants compared to other mineral fertilizers.

Tamer et al. (2016) applied humic acid and leonardite together with nitrogen and phosphorus fertilizers to the soil in their study on yield and yield components of sunflower plant. As a result of the study; they concluded that all organic materials applied together with nitrogen and phosphorus fertilizers during the experiment significantly increased yield factors such as plant height and thousand grain weight and the yield/da. They also determined that the amount of available phosphorus and organic matter in the soil increased and the soil pH decreased with the addition of organic material.

Süzer & Çulhacı (2017) investigated the effects of different organomineral and inorganic compound fertilizers on grain yield and some yield components of winter bread wheat (Triticum aestivum L.). As a result of the research; the highest grain yield in winter Selimiye bread wheat variety was determined from the application of 12N.12P.0K+12S at a dose of 25 kg/da at the base under the soil in autumn before sowing and 15 kg/da Urea at the top tillering in spring and 15 kg/da Ammonium Nitrate (33% N) at a dose of 15 kg/da in spring at the tillering stage of the plants. As a result, they suggested that a balanced fertilization program should be made in which organomineral fertilizers are used as base fertilizer and inorganic fertilizers are used as top fertilization in order to obtain high yield from unit area in winter bread wheat cultivation.

Atıcı (2020) examined the effect of organomineral and chemical fertilizer use on yield and quality characteristics of wheat plant in the 2018-2019 growing period. In the study, 20-20 and 15-15-15 base fertilizers were used as chemical fertilizers and Urea (46% N) as top fertilizer, 16-15+OM and 12-15-5+OM base fertilizers as organomineral fertilizers and 26% nitrogen containing organomineral fertilizer as top fertilizer. In terms of grain yield and biological productivity (biomass) values obtained from organomineral fertilizer and chemical fertilizer applications, the highest grain yield was obtained in organomineral fertilizer [(16-15 + OM) + (26% Nitrogen + OM)] application. In general, it was stated that organomineral fertilizer applications gave better results than chemical fertilizer applications in wheat plants.

4. STUDIES BASED ON ORGANOMINERAL FERTILIZERS IN THE WORLD

Tiwari et al. (1996) investigated the effects of organic manure and N, P, K fertilization on soil enzyme activities and microbial life in India. They stated that inorganic N, P, K fertilizer mixed with organic fertilizer has more effect on enzyme activity and microbial population than N, P, K fertilizer alone and organic fertilizer should be added in order to get the most benefit from N, P, K fertilization in soil.

Kurmysheva & Efremov (1998) conducted a study to compare the effects of mineral and organomineral fertilizers on the yield factor of potatoes, barley and alfalfa, and only alfalfa, winter wheat, potatoes, barley and corn for two years. They determined that the applied fertilizer and increasing fertilizer doses increased the effect on yield and that the best results were obtained from the fields where organomineral fertilizer was used.

Tolessa & Friesen (2001) determined that 75% of the NP inorganic fertilizer requirement to be used in maize production can be provided by farmyard manure and this will not cause any decrease in yield.

Colugnati et al. (2003) investigated the effects of organomineral fertilizer applications on grapes. As a result of the research; they stated that organomineral fertilizer applications had positive effects on grape yield, stem part development and fruit ripeness.

Tejada et al. (2005) examined the effects of organic + inorganic fertilizers and organomineral fertilizers on wheat plants. They found that the N/P ratio was higher in the soils where organomineral fertilizers were used; in addition, wheat plant showed 2.9% increase in grain protein content, 2.2% increase in the number of grains in the spike, 3.4% increase in the number of spikes per square meter, 3.9% increase in thousand grain weight and 2.5% increase in yield.

Bayu et al. (2006) determined that the NP inorganic fertilizers to be applied in sorghum production could be reduced by 50% and integrated with farmyard manure. Ghosh and Sharma (1999) and Negasa et al. (2001) argued that the integrated application of farmyard manure with inorganic NP fertilizer can result in more significant yields in rice and maize plants.

Kumari et al. (2006) examined the effects of farmyard manure and micro nutrients along with mineral fertilizers on yield components, fiber quality characteristics and economy of cotton during 2003-2004 and 2004-2005 at the agricultural research institute in the Lam region of India. In their study; they found that application of 25% or 50% farmyard manure with nitrogen, phosphorus and potassium provided increases in plant height, number of fruit branches, number of bolls and quality parameters of fibers produced in 2004-2005 season were 2.5% higher than the previous period.

Sofi et al. (2006) investigated the effects of organic and inorganic fertilization on yield and yield components of pea (Pisum sativum L. ssp. Hortense Asch and Graebn) in Kashmir region of India in 2003-2004. The treatments were inorganic fertilizers such as NPK (3:6:9 kg/da), farmyard manure (2 t/da), sheep manure (1.6 t/da), chicken manure (400 kg/da) and chemical fertilizers combined with organic fertilizers at 50-75% rates in full and half doses. Harvest maturity was 122 days in 75% chemical fertilizer + 400 kg/da chicken manure application and 121.33 days in 100% chemical fertilization.

Suryawanshi et al. (2006) studied the effects of organic (wheat straw: 500 kg/ha, farmyard manure: 500 kg/ha) and chemical (1.5-3 kg/ha N, 3-6 kg/ha P_2O_5) fertilizers on yield and yield components of soybean in India. As a result of the study, they determined that the combined use of organic and mineral fertilizers (wheat straw + farmyard manure + 5 kg $ZnSO_4$/ha) increased the number of pods, grain yield, 1000 grain weight and biological yield of the plants. Among the mineral fertilizers, the highest grain yield was obtained from 3 kg N/ha+6 kg P_2O_5/ha plots with an increase of 41%, while half dose chemical fertilizer (1.5 kg N+3 kg P_2O_5/ha) increased the grain yield by 38% with values close to full dose application. Among the organic fertilizers, the highest yield increase rate was 24% in the plots where wheat straw + barnyard manure + 5 kg $ZnSO_4$/ha fertilizer mixture was applied, while the plots where barnyard manure was applied alone were below the control.

Sulieman & Tageldin (2009) investigated the effect of farmyard manure and phosphorus fertilizer applications on bean growth and reported that farmyard manure and phosphorus fertilizer applications positively affected bean growth, but this effect was insignificant in number. They found that the application of farmyard manure together with phosphorus fertilizer had a significant effect on stem dry weight.

Gopinath et al. (2009) investigated the effects of organic fertilization (farmyard manure 2 t/da + biofertilizer + phosphorus solubilizing bacteria) and compound fertilization (farmyard manure 1 t/da + NPK 2;2.62;3.02 kg/da) on pea yields with five different pea species in India in a two-year study. As a result of the study; plant length was 45.5 and 50.2 cm in both years in the application of farmyard manure applied together with mineral fertilizers compared to the plots with organic fertilization. The number of pods per plant was 6.5-7.5, the number of grains per pod was 4.8-5.1, pod length was 7.55-7.75 cm and fresh pod yield was 14%.

Merken et al. (2012) applied three different fertilizers and three different fertilizer doses in their study in which they investigated the effects of organomineral fertilizers on the yield and quality of Sultani seedless grape variety. In the results of the two-year study; it was determined that cluster weight (g), number of clusters (pieces/bushel), yield (kg/ bushel)), grain weight (g) and water-soluble dry matter (%) contents increased especially in organomineral fertilizer applications.

Rady (2012) investigated the effects of organomineral fertilizers containing 2-10-1 kg/ha compound fertilizer + calcium sulphate and humic acid on tomato plant growth, protein content, chlorophyll content and nutrient contents of the plant. As a result of the study; It was determined that organomineral fertilizers alleviate the effects of salinity, which negatively affects plant growth, and increase the yield of tomato plants and the antioxidant content of the fruit.

Deeks et al. (2013) compared the cultivation of wheat, oilseed, barley, bean and maize plants with conventional and organomineral fertilizers. Organomineral fertilizer was obtained by drying 3-6 mm diameter domestic wastewater treatment sludge granules at 80 ^{o}C. As a result of the three-year study, it was determined that there was no significant difference in the plant yields. This result showed that organomineral fertilizers obtained from domestic wastewater treatment sludge were as effective as conventional fertilizers, and the heavy metal level did not exceed the permissible level in the soil.

Onat (2015) examined the effects of 12-12-12 and 10-25-0 organomineral fertilizers and observed the positive effects of organomineral fertilizers on yield components such as grain yield, 1000 grain weight, spike weight and stem thickness of sunflower plant. In addition, it was reported that organomineral fertilization positively affected the plant nutrient contents and yield quality factors of sunflower plant.

Almeida et al. (2023) investigated the effects of different doses of organomineral fertilizer formulations (OMF) on the growth and yield of bean plants. In the study; Organomineral fertilizer formulations were used as 0, 30, 60, 90, 120 and 150 kg/ha with and without addition of 40 kg/ha nitrogen dose. As a result of the research; morphological characteristics of beans were affected by organomineral fertilizer applications with N addition; They stated that, N and K were taken up more in the presence of organomineral fertilizer, and 1000 grain weight and grain number increased. They also determined that the highest yield was obtained at a dose of 120 kg/ha. In conclusion; they stated that the use of organomineral fertilizers is a viable alternative with great potential for soil fertilization in agricultural production, as well as environmental sustainability resulting from the inclusion of beans in the production cycle.

5. RESULTS AND RECOMMENDATIONS

Although the land available for agriculture in the world is gradually decreasing, the need to produce more food to feed the growing population has created the necessity to increase the amount of yield to be obtained from the unit area. As a result, intensive tillage, use of chemical fertilizers and pesticides has increased all over the world. However, while yield and production increase with this intensive tillage, use of chemical fertilizers and pesticides in agricultural areas, the sustainability of soil fertility and the natural balances in the soils have started to deteriorate.

Soil is a heterogeneous and dynamic system with biological activity formed from various inorganic and organic compounds under very different environmental conditions on the upper surface of the earth's crust, on which all living things live together. At the same time, soil ensures the continuity of life by providing living things with opportunities such as nutrition and shelter. However, it is seen that this system is gradually deteriorating due to intensive tillage, use of chemical fertilizers and pesticides, as well as incorrect agricultural practices. One of the most important indicators of this degradation is the decrease in the organic matter content of the soils. It is a known fact that as the organic matter content of soils decreases, biodiversity is negatively affected and soils starts to deteriorate. At this point, especially by increasing the organic matter content of soils, the quality of the soils will be improved and the system will be corrected. In the realization of these objectives, applications that can utilize organic wastes are of great importance. In addition to these applications, the use of organomineral fertilizers is a good alternative in terms of meeting the nutrients needed by cultivated plants.

Organomineral fertilizers are fertilizers that are created by combining the nutrients that the plant needs during the growing period with organic materials produced by natural formations. It contains the plant nutrients and organic matter found in chemical fertilizers together and the nutrient content can be presented in a more standardized way. In organomineral fertilizers, plant nutrient minerals such as nitrogen, phosphorus, potassium, sulfur, zinc and humic-fulvic acid and compost-derived organic matter are combined together and can be used as base fertilizer. Organomineral fertilizers produced in the form of "organic matter + mineral fertilizer" by taking advantage of the positive effects of organic materials on soil fertility, on the one hand, reduce the loss of nutrients through leaching, and on the other hand, increase the effectiveness of the minerals used by improving the fertility elements of the soil. Additionally, organomineral fertilizers can be an alternative to prevent damages in agricultural production.

It is seen that our producers use organomineral fertilizers in different ways in our country. While the majority of our producers prefer commercial organomineral fertilizers, some of them apply by mixing organic fertilizers with mineral fertilizers, and some of them use organomineral fertilizers formulated by themselves. The results of organomineral fertilizer studies carried out in our country and in the world show that organomineral fertilizers positively affect soil properties, increase yield in agricultural production, and positively affect plant growth and mineral nutrition.

As a result, fertilization is one of the important inputs for agricultural production when it is done in a balanced, sufficient and in accordance with scientific rules based on soil and plant analysis results. However, it is necessary to develop new fertilizers and alternative fertilization techniques in order to reduce the negative effects of chemical fertilizers used intensively and to increase the organic matter content of the soil. Organomineral fertilizers seem to be a good alternative in this respect. However, in order for the use of organomineral fertilizers to become widespread, producers need to be informed, raised awareness and supported.

REFERENCES

Akıncı, C., Yıldırım, M.,Doran, D., & Akçan, A. (2007). Ekmeklik buğdayın verim ve verim unsurları üzerine tescilli organomineral gübrelerin etkileri. Türkiye VII. Tarla Bitkileri Kongresi 25-27 Haziran 2007, Erzurum.

Almeida, V., Carneiro, G., Almeida, R., Souza, B., Teixeira, I., Vieira, J., & Leandro, W. (2023). Development and yield of common bean in response to organomineral fertilization based on filter cake., Journal of Plant Nutrition 46 (14): 3292-3311.

Anonymous. (2003). Tarımda kullanılan organik, organomineral, toprak düzenleyicileri ve mikrobiyal gübrelerin üretimi, ithalatı, ihracatı, Ppiyasaya arzı ve denetimine dair yönetmelik. Resmi Gazete Tarihi: 22.04.2003 Resmi Gazete Sayısı: 25087.

Anonymous. (2017a). Annexes to the proposal for a regulation of the European Parliament and of the council laying down rules on the making available on the market of ce marked fertilising Products and Amending Regulations (EC) No 1069/2009 and (EC) No 1107/2009. Erişim adresi:https://ec.europa.eu/transparency/regdoc/rep/1/2016/en/1-2016-157-en-f1-1-annex-1.pdf.

Anonymous. (2017b). Tarımda kullanılan organik, organomineral gübreler ve toprak düzenleyiciler ile mikrobiyal, enzim içerikli ve organik kaynaklı diğer ürünlerin üretimi, ithalatı, ihracatı ve piyasaya arzına dair yönetmelik. Resmi Gazete Tarihi: 29.03.2014 Resmi Gazete Sayısı: 28956.

Anonymous. (2018). Organomineral gübre çalıştayı bildiriler kitabı (Editör: E. Kınacı), 1. Basım: Düzenleyen: TEMA, SÜTAŞ, ISBN: 978-975-7169-89-5. Sena Ofset Ambalaj Matbaacılık, İstanbul.

Anonymous. (2021). Muğla ili entegre biyogaz ve organomineral gübre üretim tesisi ön fizibilite raporu. T.C. Sanayi ve Teknoloji Bakanlığı, Güney Ege Kalkınma Ajansı, 33 syf)

Atıcı, C.T. (2020). Kimyasal ve organomineral gübre uygulamasının buğday bitkisinin verim ve bazı kalite özellikleri üzerine etkisi. Kahraman Maraş Sütçü İmam Üniversitesi Fen Bilimleri Enst., Toprak Bilimi ve Bitki Besleme ABD, Yüksek Lisans Tezi, 22 syf.

Bayu, W.N., Rethman, F. G., Hammes P. S., &Alemu. G. (2006). Application of farmyard manure improved the chemical and physical properties of the soil in a semi-arid area in Ethiopia," Biological Agriculture and Horticulture, Vol.24, No.3, pp.293-300. doi: 10.1080/01448765.2006.9755027

Çalışkan, Ö. & Ayan, A.K. (2011). Isırganda (Urtica dioica L.) farklı dozlarda NPK"lı organomineral gübrelerde verim ve verim komponentlerine etkisi, Anadolu Tarım Bilim. Dergisi, 26(3),217-220.

Colugnati, G., Crespan, G., Pıcco, D., Bregant, F. & Gallas, A. (2003). Effects of agronomineral fertilizers and a plant growth regulator on grape, Informatore Agrario Journal, 59 (24), 67-70 pp.

Demirtaş, E.I., Özkan, C.F., Arı, N., & Asri, F.Ö. (2012). Bazı organik ve kimyasal gübre uygulamalarının domateste verim ve kalite üzerine etkileri. Alatarım Dergisi 2012, 11 (2), 9-16.

Deeks, L. K., Chaney, K., Murray, C., Sakrabanı, R., Gedara, S., Le, M. S., Tyrrel, S., Pawlett, M., Read, R. & Smıth, G. H. (2013). A new sludge-derived organo-mineral fertilizer gives similar crop yields as conventional fertilizers, Agronomy for Sustainable Development, 33 (3), 539-549.

Erdal, İ., Bozkurt, M. A., & Çimrin, K.M. (2000). Hümik asit ve fosfor uygulamalarının mısır bitkisinin (Zea mays L.) Fe, Zn, Mn ve Cu içeriği üzerine etkisi, A. Ü. Z. F. Tarım Bilimleri Dergisi 6 (3) 91-96.

Erdal, İ. (2018). Türkiye'de organomineral gübrelerin kullandığı araştırma çalışmaları ve elde edilen sonuçlar, Organomineral Gübre Çalıştayı, 156-165, İstanbul.

Gopinath, K.A., Saha, S., Mina B.L., Pande H., Kumar, N., Srivastva, A.K., & Gupta, H.S. (2009). Yield potential of garden pea (Pisum sativum L.) varieties, and soil properties under organic and integrated nutrient management systems. Archives of Agronomy and Soil Science, 55 (2), 157–167.

Günay, A. (2014) Organomineral gübre uygulamalarının ayçiçeğinin verim ve kalite parametreleri üzerine etkileri. Yüksek Lisans Tezi.Ege Üniversitesi, Fen Bilimleri Enstitüsü, Toprak Bilimi ve Bitki Besleme Anabilim Dalı. İzmir.

Iğdırlı, D. (2006). Adana koşullarında organik çilek fidesi yetiştirme olanakları. Yüksek Lisans Tezi, Ç.Ü., Fen Bilimleri Enstitüsü, Bahçe Bitkileri Anabilim Dalı, Adana.

Kacar, B., Katkat, V., 2021. Gübreler ve gübreleme tekniği, Nobel Akademik Yayıncılık.

Karaca, A., Turgay, O.C. & Tamer, N. (2005) Effects of humic deposit (gyttja) on soil chemical and properties and availability of heavy metal in soil. Biology and Fertility of Soils 42 (6): 585-592.

Karaman, M.R. (2012). Bitki besleme (Editör: M.R. Karaman), Gübretaş Rehber Kitapları Dizisi:2, 685-729, Ankara.ISBN:978-605-87103-2-0.

Kominko, H., Gorazda, K. & Wzorek, Z. (2016). The possibility of organo-mineral fertilizer production from sewage sludge, Waste Biomass Valor, DOI: 10.1007/s12649-016-9805-9.

Kumari, S. R. & Subbaramamma, P., (2006). Effect of farm yard manure, chemical fertilizers and micronutrients on yield, economics and fibre properties of cotton. Journal of Cotton Research and Development, 20(1), 64-70.

Kurmysheva, N. A. & Efremov, V. F. 1998. The effect of saturation of the system of fertilization of rotations with organic fertilizers on the yield of agricultural crops and quality of production in conditions of the Moscow Region, Agrokhimiya journal, 8: 26-32.

Merken, Ö., Ünal, A., İnan, M.S., & Karabat, S. (2012). Sultani çekirdeksiz üzüm çeşidinde farklı form ve dozdaki organomineral gübrelerin verim ve kaliteye olan etkileri üzerine bir araştırma, Manisa Bağcılık Araştırma Enstitüsü organomineral gübre raporu.

Onat, M. (2015). Organomineral gübre uygulamasının ayçiçeği verim ve kalite parametreleri üzerine etkisi. Yüksek lisans tezi, Ege Üniversitesi, Ziraat Fakültesi, Fen Bilimleri Enstitüsü, Toprak Bilimi ve Bitki Besleme Anabilim Dalı, İzmir.

Özalp, M. (2010). Geleneksel gübreleme ile farklı organik gübre kaynaklarının tir buğday‟ında (Triticum aestivum L. var. Leucospermum (körn.) farw.) verim ve bazı verim öğeleri üzerine etkisi (yüksek lisans tezi, basılmamış). Y.Y.Ü. Fen Bilimleri Enstitüsü, Van.

Özcan, F., Erekul,O & Konak,C. (2004). Farklı organik ve mineral azot gübrelemesinin ekmeklik buğdayda protein ve aminoasit miktarına etkisi. Türkiye 3. Ulusal Gübre Kongresi. Tarım-Sanayi-Çevre. S: 391-398. 11-13 Ekim 2004, Tokat.

Özkan, C.F., Arı, N., Asri, F.Ö., & Demirtaş, E.I. (2012). Örtü altı biber yetiştiriciliğinde organik ve kimyasal gübre uygulamalarının bitkinin beslenme durumu ve bitki gelişimi üzerine etkileri. 6. Ulusal Bitki Besleme ve Gübre Kongresi 03-07 Haziran Nevşehir.

Özkan, U. (2013). Bazı azotlu ve organomineral gübrelerin çok yıllık çim (Lolium perenne K.)'de kalite ve gelişimine etkileri. Ankara Üniversitesi, Fen Bilimleri Enstitüsü, Tarla Bitkileri Ana Bilim Dalı, Yüksek Lisans Tezi.

Pekcan, T., Turan, H. S., & Çolakoğlu, H. (2009). Effects of organomineral, mineral and farm yard manures on the yield and quality of olive trees (Olea europaea L.). In The Proceedings of the International Plant Nutrition Colloquium XVI.

Preusch, P.L., Adler, P.R., Sikora, L.J., & Tworkoski, T.J. (2002). Nitrogen and phosphorus availability in composted and uncomposted poultry litte. Journal of Environmental Quality 31 (6): 2051-2057.

Rady, M. M. (2012). A novel organomineral fertilizer can mitigate salinity stress effects for tomato production on reclaimed saline soil agricultural. Botany department, Faculty of Agriculture, Fayoum University, 63514-Fayoum, Egypt.

Sağlam, M. T., Özel, E. Z., & Bellitürk, K. (2012). İki farklı tekstüre sahip toprakta leonardit organik materyalinin mısır bitkisinin azot alınımına etkisi. SAÜ Fen Edebiyat Dergisi, (1). 383-391.

Shan, L., Yunheng, H., Chen, J., Huang Q., Lian, X., Wang,H.&Liu, Y. (2015). Nitrogen surface runoff losses from a Chinese cabbage field under different nitrogen treatments in the Taihu Lake Basin, China, Agricultural Water Management 159: 255-263.

Sofi, A. H., Singh, A. K., Ahmed, N., Narayan, S. & Shahnaz. M. (2006). Effect of organic manures and inorganic fertilizers on maturity growth, yield and economics of garden pea cv Azad P-1 (Pisum sativum L. ssp. hortense Asch and Graebn). Environment and Ecology 24 (3A), 857-859.

Sulieman, S. A., & Tageldin E. M. H. (2009). The Effects of Phosphorus and Farmyard Manure on Nodulation and Growth Attributes of Common

Bean (Phaseolus vulgaris L.) in Shambat Soi lunder Irrigation. Research Journal of Agriculture and Biological Sciences, 5 (4), 458-464.

Suryawanshi, S.B., Lad, N.G., Suryawanshi V.P., Shaikh A.K., & Adsul, P.B. (2006). Combined effect of organic and inorganic fertilization on yield attributes and yield of soybean (Glycine max. L. Merrill). J. Soils and Crops, 16(1), 145-147.

Süzer, S. & Çulhacı, E. (2016). Effects of different organomineral and inorganic compound fertilizers on seed yield and some yield components of sunflower (Helianthus annuus L.). 19 th International Sunflower Conference, Edirne, Turkey, 2016.

Tamer, N., Başalma, D., Türkmen, C., & Namlı, A. (2016). Organik toprak düzenleyicilerin toprak parametreleri ve ayçiçeği (Helianthus annuus L.) bitkisinin verim ve verim öğeleri üzerine etkileri. Toprak Bilimi ve Bitki Besleme Dergisi 4: 11-20.

Tejada, M., Benıtez, C. & Gonzalez, J. L. (2005). Effects of application of two organomineral fertilizers on nutrient leaching losses and wheat crop, Agronomy journal, 97 (3), 960.

Tisdale, S.L. and Nelson, W.L. (1982) Fertilidad de los suelos y fertilizantes. Ed. UTHEA, México, 760 p.

Tiwari, S.C. (1996). Effects of organic manure and N P K fertilization on enzyme activities and microbial populations in substropical oxisol, 9(2), 334-340.

Tolessa, D., & Friesen, D.K. (2001). Effect of enriching farmyard manure with mineral fertilizer on grain yield of Maize at Bako, Western Ethiopia. Seventh Eastern and Southern Africa Regional Maize Conference. 11th-15th February. 335-337.

Tunç, N. (2017). Avrupa birliği'nde kullanılan biyolojik gübreler ve biyolojik gübre kullanım potansiyelinin belirlenmesi, T.C. Gıda, Tarım ve Hayvancılık Bakanlığı, AB Uzmanlık Tezi, Ankara.

Yazıcı, M.A. (2001). Sera koşullarında toprağa uygulanan Gidya‟nın buğdayın büyümesi, yeşil aksamı, bor ve çinko konsantrasyonu üzerine etkisi. Yüksek lisans tezi, Çukurova Üniversitesi, Adana.

Yıldız, N. (2018). Mineral gübrelerin toprak ekosistemi, çevre ve bitkisel üretim üzerine olası etkileri, Organomineral Gübre Çalıştayı, 212-243, İstanbul.

Zengin, M., Gökmen, F., & Gezgin, S. (2010). Kimyasal gübreler hümik asit uygulamaların ıspanakta verim ve verim unsurlarına etkileri. Türkiye IV. Organik Tarım Sempozyumu. 28 Haziran-1 Temmuz 2010, Erzurum.

CHAPTER 4

NANOTECHNOLOGY FOR SUSTAINABLE AQUACULTURE

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1.INTRODUCTION

The methodology of the United Nations (UN) projected a global population of around 9 billion by 2050 and 11.2 billion by 2100. Hence, the endeavor to meet the increasing worldwide need for food is getting more difficult and requiring the investigation of novel food resources. Given the limited accessibility of land and water resources on a global scale, it is clear that a future time of competition for these resources is inevitable. Aquaculture production have the capability to satisfy the growing demands of the human population (Naylor et al., 2021). Environmental problems, including as eutrophication, climate change, and water pollution, hinder the growth of aquaculture. High temperatures and dry circumstances affect the water quality, the makeup of plankton communities, and the reproductive ability of fish. Contaminated rivers result in the presence of pollutants and high levels of toxicity, which in turn cause chronic nutritional insufficiency in embryonic and breeding fish populations. Managing fish health is a complicated effort, since it requires addressing prevalent infections and diseases such as fin and gill infection and EUS. The aquatic organism industry has seen financial setbacks and the rise of antibiotic resistance, resulting in uncertainties over medical strategies.Traditional methods of administering medicine and diagnosing illnesses are ineffective. Researchers are continually seeking innovative technological solutions to address the challenges encountered in aquaculture (Shah et al., 2020).

Nanotechnology is an expanding discipline that investigates both organic and inorganic substances at the nanoscale, with a primary focus on nanoparticles. It finds uses in many domains such as human, animal, plant, and environmental health, bioengineering, electronics, nuclear energy, fuel, and medicine. Nevertheless, there is a dearth of knowledge about aquaculture, a pivotal factor in supplying animal protein for human nourishment. Acquired infections and pathogens in aquatic ecosystems impede the development of nourishing aquatic commodities. The objective of this research is to investigate the future direction of nanotechnology in aquaculture, with a focus on inorganic nanoparticles and their many uses, including aquatic animal nutrition and the development of antibacterial, antibiofilm, and photocatalytic agents.Significant research exists about the use of inorganic nanoparticles for water treatment and animal feed. Additionally, there is an ongoing debate over the possible hazards linked to aquaculture. Nanotechnology is anticipated to enhance fish health, regulate dangerous bacteria, and facilitate the delivery of aquatic goods at the nano-scale in the future of aquaculture(Sarkar et al., 2022).

2.NANOPARTICLES DEFINED

Nanostructures, according to the National Nanotechnology Initiative (NNI), are deliberately designed groupings of materials that have at least one dimension of 100 nm or less. Nanomaterials (NMs) and nanoparticles (NPs) do not have a widely agreed upon technical definition. Nevertheless, nanomaterials (NMs) are often defined to be substances has sizes varied at surrounding 1 nm to 100 nm.The compounds in question are often known as nanoparticles, nanowires, and nanotubes. These compounds may exist in many forms, including one-dimensional, two-dimensional, or multidimensional structures (Curda et al., 2023).

1.1. Applications of Nanoparticles

Nanotechnology's distinct physical and chemical characteristics have attracted a lot of interest from a variety of businesses. Common carbon-based particles used in a variety of fields, including mechanical engineering, biomedicine, and nanoelectronics, include carbon nanotubes, fullerenes, carbon black, nanodiamonds, graphite nanoparticles, and graphene oxide. Because of its plasmonic, photothermal, stability, high reactivity, and physiochemical characteristics, metallic nanoparticles are widely used. Micelles, liposomes, dendrimers, and polymers are examples of organic-based nanoparticles that find use in drug delivery, chemical sensing, biosensing, diagnostics, polymer materials, and pharmaceuticals that treat prion-related diseases.Significant advancements in biological and pharmaceutical applications are made possible by the notable physical contrasts between nanomaterials and traditional materials. The creation of a wide variety of nanomaterials and the alteration of their chemical, physical, and perhaps biological characteristics for medical applications have been made possible by advances in nanotechnology. More possibility exists for accurate and quick diagnosis as well as efficient illness treatment with few adverse effects thanks to nanotechnology (Patel et al.,2019). Because they may enter tissues and cells more readily and travel more readily than bigger molecules, nanomaterials are more likely than larger particles to harm unwanted species. Research has shown a link between nanoparticles and detrimental cell effects, including cytokine production, oxidative stress, and DNA damage. Reactive oxygen species (ROS) are produced when cells absorb silver nanoparticles, and this may lead to oxidative stress and genotoxicity(Handy et al., 2008;Turan and Ergenler, 2023).

A new field of study called nanotoxicology looks at the toxicological properties of artificial nanoparticles used in biomedicine. Gaining insight into how nanoparticles interact with living things or the environment is essential to comprehending the possible medical advantages (Tüylek, 2017; Ergenler et al., 2023).

3.NANOTECHNOLOGY IN FISH FEEDING

When introduced to fish's diet, nanostructures may act as growth-promoting and immune-modulating substances. In crucian carp, for example, the use of selenium nanoparticles may improve cell metabolism, encourage weight increase, and alter the reactive profile. In comparison to metals selenium, nanoscale selenium exhibits greater control of seleno-enzymes and less toxicity. With zebrafish (*Danio rerio*), nanosilver particles increases the amount of activity of proteolytic and metalloprotease enzymes, which boosts growth overall. Furthermore, beneficial growth patterns are observed in both common carp (*Carassius auratus gibelio*) and grass carp (*Ctenopharyngodon idella*) upon the injection of zinc oxide and selenium nanoparticles.Nanoparticles of magnesium oxide enhance the growth of post-larvae that are fed crustaceans (*Macrobrachium rosenbergii*). Sarkar et al. (2022) shown that small particles of zinc improve the growth and immunomodulation of catfish (*Pangasius hypophthalmus*) in situations with both stress caused by abiotic and biotic factors.

4. NANOMEDICINE

Nanomedicine is the use of nanotechnology in clinical and animal health. It involves the development and utilization of nanoparticles and nanodevices for biomedical purposes. Disease outbreaks pose the most significant obstacles to the growth and long-term viability of aquaculture.Nanotechnology is important in providing novel perspectives on the diagnosis and treatment of diseases (Handy, 2008).

Dendrimers, liposomes, carbon nanotubes, nanospheres, and nanocapsules are some of the minuscule particles used in medicine. Nanospheres, also, characterized by their minute size along with expansive covered region, were fundamental in both processes of cell renewal and medication transportation. The inside core of nanocapsules contains a specific drug, while the exterior shell is formed of the surrounding material. Carbon nanotubes are ideal for delivering medicines, especially anti-tumor drugs, because of their extensive surface area and ability to easily cross cell membranes. Dendrimers are nanostructures with a complex, highly branched surface that serves several purposes. These particles may serve as carriers for antibacterial agents, genes, vaccines, pharmaceuticals, and cell regeneration. Nanoparticles have been used in aquaculture medicine primarily as antibacterial agents, pharmaceuticals, and carriers for gene delivery, vaccination, and accurate detection of fish diseases (Wu et al., 2015; Shaalan et al., 2016). The graphic illustrates the use of nanotechnology for illness control in fish in Figure 1.

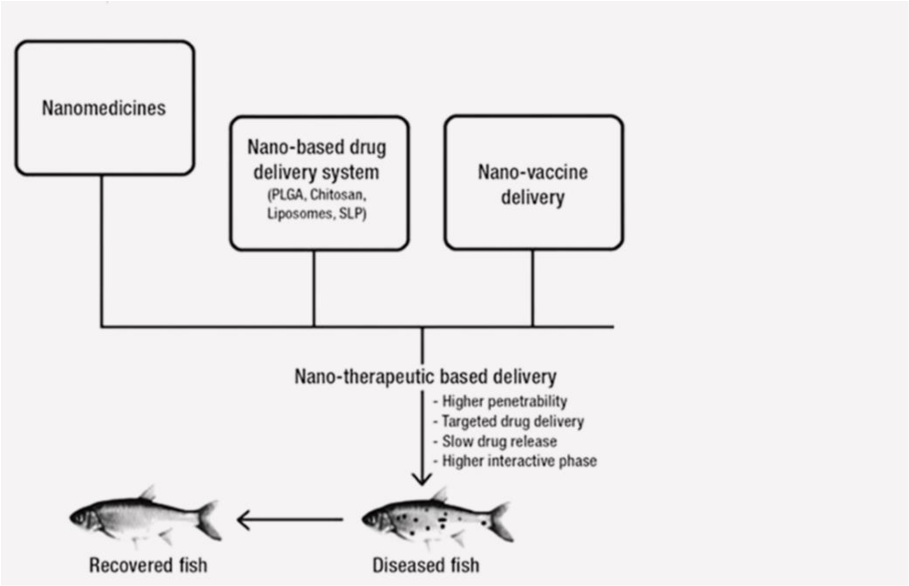

Figure 1. Nanotechnology for illness control in fish (modified Sarkar et al., 2022).

4.1. Nanovaccination on The Aquaculture

Vaccinations play a vital role in aquaculture protecting the host against diseases caused by pathogens.Conventional techniques such as injections or oral treatment have deleterious consequences and are fatal to fish. In response to this issue, nano-delivery methods have been suggested as a safer and more effective alternative. Nanovaccines are an innovative kind of vaccination that merges a pathogen-specific antigen with nanoparticles to activate the immune system against the disease. Nanoparticles possess a higher surface-to-volume ratio compared to typical macromolecules, enabling them to overcome their constraints. This characteristic enables nanoparticles to effectively target immune cells, extend the length of circulation in the body, and enhance metabolic activity in lymphoid tissues. In order to enhance the presentation of antigens, the dispersion of biological substances, and the transportation of drugs inside cells, it is possible to alter the physio-chemical characteristics of nano-carriers. Due to the rising prevalence of aquaculture-related illnesses worldwide, there is a substantial need for immunizations based on nanoparticles (Ji et al., 2015; Vartak and Sucheck, 2016; Bharadwaj et al., 2020).

Polymeric nanoparticles, composed of polymers that can be broken down naturally and are compatible with living organisms, may be used to enclose fish vaccine antigens using several methods. Due to their customized surface qualities and very tiny size, these particles may retain antigens for an extended period of time and are resistant to degradation. Oral vaccination of fish often involves the administration of organic nanoparticles that include polymers such hyaluronic acid, alginate, and chitin. This is done to improve their mucosal immunity. Previous studies have used artificial polymers, namely PLGA and PLA, in the development of vaccines for human application (Fredriksen et al., 2011; Thompson et al., 2023).

The benefits of using nanoliposomes in fish vaccines lie in their capacity to specifically target certain immune cells, their lipid-based constitution, and their distinctive form. Nanoemulsions, being stable nanotechnology formulations, have high efficacy in encapsulating various antigens and maintaining their integrity throughout storage and vaccination delivery.Immune cells uptake antigens from ISCOMs, leading to the intracellular assembly of cage-like structures.This procedure increases the ability to provoke an immune response. Carbon nanotubes (CNTs) improve the display of the antigenic target inside the fish's immune system by acting as a supportive structure for it. Virus-like particles (VLPs) are artificially created nanoparticles that imitate diseases and trigger a strong immune response without causing actual illness. They have a similarity to viruses, although they lack viral genetic material.These minuscule particles are considered suitable choices for providing comprehensive protection and have the capacity to be used due to their safety and ability to stimulate an immune response (Rosalez-Mendoza, Gonzalez-Ortaga, 2019).

5. NANOPARTICLE TOXICITY

An evaluation of nanotoxicity is crucial in order to investigate the effects of various nanostructures on the circuits of aquatic organisms, and develop safety standards for their use in aquatic ecosystem (Ergenler et al.,2023). Furthermore, they have furnished mortality data of experimental organisms that conform to regulatory ecotoxicology standards and might be used to enhance aquaculture administration. The toxicity of nanoparticles is influenced by their dimensions, shape, content, size distribution, porosity, and surface charge. These properties also impact the way in which the nanoparticles are taken in and react to physiological fluids. Typically, the interactions follow this sequence: positively charged particles exert a stronger influence than negatively charged particles, which in turn exert a stronger influence than neutrally charged particles. Nevertheless, the order of this sequence may vary based on the specific nanoparticle of interest.The stability of a substance is determined by several elements, such as the toxicity of the ions generated after breakup, the level of the fact, its surface area, the process of agglomeration, and the performance of its surface. The morphology of nanoparticles significantly influences their uptake and cellular availability. The immobilization of nanoparticles by the coating medium is equally crucial in toxicological study (McCracken et al., 2016).

Metallic nanoparticles are used in targeted medical treatments to combat antibiotic-resistant illnesses. However, the correlation between reactive oxygen species activity and toxicity can be paradoxical, requiring further investigation. Nanoparticles can be potentially dangerous in various ways, including DNA damage, and their toxicological impacts can be investigated through in vitro and in vivo tests. Techniques can be used to analyze the physicochemical and structural features of nanoparticles to determine their level of toxicity(Yochabedh et al., 2023).

Reactive oxygen species can be generated by metals and metal oxide nanoparticles, as well as metallic surfaces, through surface-related processes such as photoexcitation, corrosion, and catalysis. Metal ions and NPs can participate in Fenton-like reactions, transforming H_2O_2 into harmful radicals. These interactions can disturb the balance of reactive oxygen species in cells and induce toxicity.The band gap characteristics and toxic strength of metal oxide nanoparticles are known to interact with biological oxidative processes. Metal oxide nanoparticles can disrupt the equilibrium of reactive oxygen species in cells, potentially causing damage to mitochondria even without electrical activity. A carbon nanotube and NADH peroxidase bioelectrode array can be used to measure the production of H_2O_2 in NPs in non-living environments, assessing the generation of reactive oxygen species (Zhang et al., 2012; Lakshmi et al., 2015; Gilbert et al., 2017).

5.1.The Adverse Effects of Nanomaterials in Model Organisms

In previous a long time, much investigation has taken conducted using living species along with laboratory models to investigate the possible impacts of different nanoparticles (NPs), with nothing or any focus for that form about these nanoparticles (Ergenler et al., 2023 ; Alaraby et al., 2020; Demir 2014; Demir et al., 2015;; Demir and Castranova, 2016; Demir et al., 2017, Demir & Marcos, 2018; Domenech et al., 2020; Demir, 2020Habas et al., 2018; Huang et al., 2013; Tokgun et al., 2015; Vales et al., 2013). Ergenler et al., (2023), The researchers said that researchers manufactured bismuth sulfide (Bi_2S_3) nanoflowers (NFS) via a hydrothermal treatment technique focused on waves. They then examined the gene-toxic consequences with these nanoflowers to *Cyprinus carpio*. Based on the European Regulations 93/67/EEC, the categorization system indicates that it is non-hazardous. Other studies Categorization of nanostructures based on their average size Concentrate(s) Cellular lines and organism tests are used to assess the toxicity and evaluate the potential for genetic harm. Citations of the findings Gallium Phosphide Nanowires: The concentration of 40 nm nanoparticles in the solution was found to be 6.2 ± 1.6 x 1010 nanowires per liter, as measured in *Daphnia magna*. There were no deaths recorded in the group of D. magna that were exposed to GaPNWs, as well as in the control group. The results indicate that the feeding behavior of specimens may enhance the ability of NWs to navigate cellular barriers, with the width of the NWs playing a critical part in this process. Liu et al. (2014) conducted a study using Al_2O_3 nanowires exhibit diameters ranging from 2 to 6 nanometers and lengths ranging from 200 to 400 nanometers. The available concentration levels for the nanowires are 50, 100, or 200 milligrams per milliliter. Hashimoto et al., (2016). The L929 tissues and RAW264 mice macrophages cultures were analyzed using WST-8 and LDH assays. The viability of the cells was unaltered after exposure to different doses of Al_2O_3 NWs. The LDH release did not exhibit a significant increase when L929 and RAW264 cells were exposed to Al_2O_3. The Al_2O_3 nanowires (NWs) exhibited no cytotoxicity and did not induce damage to the cellular nuclei in RAW264 cells. In addition to CeO_2 nanorods with lengths more than or equal to 200 nm and aspect ratios greater than or equal to 22. Another kind of hematopoietic cell lines produced from human cells. The user has provided the text "(THP-1)". Addition that CeO_2 nanoparticles have been shown to exhibit a progressive rise in inflammation. Cellular damage is caused by reactions.Several studies have explicitly investigated the toxicity of nanomaterials (NMs) and their impact on DNA damage (Alaraby et al., 2016; Kermanizadeh et al., 2016).

6. CONCLUSION

The field of nanotechnology science and advancement presents many hurdles, along with distinct and abundant potential to enhance and revolutionize conventional aquaculture operations. Furthermore, the advantages of nanotechnologies are worthy exploring, and its major knowledge on the risks is derived from the apply of nanotechnology in environmentally friendly, advanced the aquaculture sector. The significant advancements introduced by nanomaterials also give rise to issues. Hence, there currently is a need to enhance toxicology investigations on the nanotoxicity process in order to develop efficacious fish vaccines. (Kheirollahpour et al., 2020).

REFERENCES

Alaraby, M., Annangi, B., Marcos, R., & Hernández, A. (2016). Drosophila melanogaster as a suitable in vivo model to determine potential side effects of nanomaterials: A review. Journal of Toxicology and Environmental Health, Part B, 19(2), 65–104. https://doi.org/10.1080/10937404.2016.1166466

Alaraby, M., Annangi, B., Marcos, R., & Hernández, A. (2016). Drosophila melanogaster as a suitable in vivo model to determine potential side effects of nanomaterials: A review. Journal of Toxicology and Environmental Health, Part B, 19(2), 65-104.

Alaraby, M., Demir, E., Domenech, J., Velázquez, A., Hernández, A., & Marcos, R. (2020). In vivo evaluation of the toxic and genotoxic effects of exposure to cobalt nanoparticles using Drosophila melanogaster. Environmental Science: Nano, 7(2), 610–622. https://doi.org/10.1039/C9EN00690G

Bhardwaj, P., Bhatia, E., Sharma, S., Ahamad, N., & Banerjee, R. (2020). Advancements in prophylactic and therapeutic nanovaccines. *Acta biomaterialia*, *108*, 1-21.

Curda, P., Kaftan, D., Kozak, T., Kumar, S., Sezemský, P., & Stranak, V. (2023). High-defined and size-selective deposition of nanoparticles by their manipulation in an electrostatic field. Applied Surface Science, 640, 158307.

Demir, E. (2020). An in vivo study of nanorod, nanosphere, and nanowire forms of titanium dioxide using Drosophila melanogaster: toxicity, cellular uptake, oxidative stress, and DNA damage. Journal of Toxicology and Environmental Health, Part A, 83(11-12), 456-469.

Demir, E., & Castranova, V. (2016). Genotoxic effects of synthetic amorphous silica nanoparticles in the mouse lymphoma assay. *Toxicology Reports*, *3*, 807-815.

Demir, E., & Marcos, R. (2018). Toxic and genotoxic effects of graphene and multi-walled carbon nanotubes. Journal of Toxicology and Environmental Health, Part A, 81(14), 645-660.

Demir, E., Akça, H., Kaya, B., Burgucu, D., Tokgün, O., Turna, F., … Marcos, R. (2014). Zinc oxide nanoparticles: Genotoxicity, interactions with UV-light and cell-transforming potential. Journal of Hazardous Materials, 264, 420–429. https://doi.org/10.1016/j.jhazmat.2013.11.043

Demir, E., Akça, H., Turna, F., Aksakal, S., Burgucu, D., Kaya, B., … Marcos, R. (2015). Genotoxic and cell-transforming effects of titanium dioxide nanoparticles. EnvironmentalResearch, 136, 300–308.

Domenech, J., Hernández, A., Demir, E., Marcos, R., & Cortés, C. (2020). Interactions of graphene oxide and graphene nanoplatelets with the in vitro Caco-2/HT29 model of intestinal barrier. Scientific Reports, 10(1), 2793. https://doi.org/10.1038/s41598-020-59755-0

Ergenler, A., Turan, F., Zaman, B. T., Tezgin, E., Bakirdere, S., & Depci, T. (2023). Novel data on genotoxic assessment of bismuth sulfide nanoflowers in common carp Cyprinus carpio. *Environmental Monitoring and Assessment*, *195*(9), 1055.

Fisheries, F. A. O. (2006). *The state of world fisheries and aquaculture 2006*.

Fredriksen, B. N., & Grip, J. (2012). PLGA/PLA micro-and nanoparticle formulations serve as antigen depots and induce elevated humoral responses after immunization of Atlantic salmon (Salmo salar L.). Vaccine, 30(3), 656-667.

Ganguly, P., Breen, A., & Pillai, S. C. (2018). Toxicity of nanomaterials: Exposure, pathways, assessment, and recent advances. *ACS Biomaterials Science & Engineering*, *4*(7), 2237-2275.

Garlock, T., Asche, F., Anderson, J., Ceballos-Concha, A., Love, D. C., Osmundsen, T. C., & Pincinato, R. B. M. (2022). Aquaculture: The missing contributor in the food security agenda. Global Food Security, 32, 100620.

Gilbert, J. L. (2017). Corrosion in the human body: metallic implants in the complex body environment. Corrosion, 73(12), 1478-1495.

Habas, K., Abdulmwli, M., Demir, E., Jacob, B. K., Najafzadeh, M., & Anderson, D. (2018). DNA damage protection by bulk and nano forms of quercetin in lymphocytes of patients with chronic obstructive pulmonary disease exposed to the food mutagen 2-amino-3-methylimidazo [4, 5-f] quinolone (IQ). Environmental research, 166, 10-15.

Handy, R. D., Von der Kammer, F., Lead, J. R., Hassellöv, M., Owen, R., & Crane, M. (2008). The ecotoxicology and chemistry of manufactured nanoparticles. Ecotoxicology, 17, 287-314.

Hashimoto, Y., Seniutinas, G., Balčytis, A., Juodkazis, S., & Nishijima, Y. (2016). Au-Ag-Cu nano-alloys: tailoring of permittivity. *Scientific reports*, *6*(1), 25010.

Heim, J., Felder, E., Tahir, M. N., Kaltbeitzel, A., Heinrich, U. R., Brochhausen, C., ... & Brieger, J. (2015). Genotoxic effects of zinc oxide nanoparticles. Nanoscale, 7(19), 8931-8938.

Huang, L. H., Sun, X. Y., & Ouyang, J. M. (2019). Shape-dependent toxicity and mineralization of hydroxyapatite nanoparticles in A7R5 aortic smooth muscle cells. Scientific Reports, 9(1), 18979. https://doi.org/10.1038/s41598-019-55428-9.

Ji, J., Torrealba, D., Ruyra, À., & Roher, N. (2015). Nanodelivery systems as new tools for immunostimulant or vaccine administration: targeting the fish immune system. Biology, 4(4), 664-696.

Ji, Z., Wang, X., Zhang, H., Lin, S., Meng, H., Sun, B., … Zink, J. I. (2012). Designed synthesis of CeO2 nanorods and nanowires for studying toxicological effects of high aspect ratio nanomaterials. ACS Nano, 6(6), 5366–5380. https://doi.org/10.1021/nn3012114

Joosten, P. H. M., Tiemersma, E., Threels, A., Caumartin-Dhieux, C., & Rombout, J. H. W. M. (1997). Oral vaccination of fish againstVibrio anguillarumusing alginate microparticles. Fish & Shellfish Immunology, 7(7), 471-485.

Kermanizadeh, A., Gosens, I., MacCalman, L., Johnston, H., Danielsen, P. H., Jacobsen, N. R., ... & Stone, V. (2016). A multilaboratory toxicological assessment of a panel of 10 engineered nanomaterials to human health—ENPRA project—the highlights, limitations, and current and future challenges. Journal of Toxicology and Environmental Health, Part B, 19(1), 1-28.

Kermanizadeh, A., Gosens, I., MacCalman, L., Johnston, H., Danielsen, P. H., Jacobsen, N. R., ... & Stone, V. (2016). A multilaboratory toxicological assessment of a panel of 10 engineered nanomaterials to human health—ENPRA project—the highlights, limitations, and current and future challenges. *Journal of Toxicology and Environmental Health*, *Part B*, *19*(1), 1-28.

Kessler, A., Hedberg, J., Blomberg, E., & Odnevall, I. (2022). Reactive oxygen species formed by metal and metal oxide nanoparticles in physiological media—a review of reactions of importance to nanotoxicity and proposal for categorization. Nanomaterials, 12(11), 1922.

Kheirollahpour, M., Mehrabi, M., Dounighi, N. M., Mohammadi, M., & Masoudi, A. (2020). Nanoparticles and vaccine development. *Pharmaceutical nanotechnology*, *8*(1), 6-21.

Kheirollahpour, M., Mehrabi, M., Dounighi, N. M., Mohammadi, M., & Masoudi, A. (2020). Nanoparticles and vaccine development. *Pharmaceutical nanotechnology*, *8*(1), 6-21.

Lakshmi Prasanna, V., & Vijayaraghavan, R. (2015). Insight into the mechanism of antibacterial activity of ZnO: surface defects mediated reactive oxygen species even in the dark. *Langmuir*, *31*(33), 9155-9162.

Liu, L., Sun, M., Li, Q., Zhang, H., Alvarez, P. J., Liu, H., & Chen, W. (2014). Genotoxicity and cytotoxicity of cadmium sulfide nanomaterials to mice: Comparison between nanorods and nanodots. Environmental Engineering Science, 31(7), 373–380. https://doi.org/10.1089/ees. 2013.0417

Liu, Q., Gu, F., Lu, X., Liu, Y., Li, H., Zhong, Z., ... & Su, F. (2014). Enhanced catalytic performances of Ni/Al2O3 catalyst via addition of V2O3 for CO methanation. *Applied Catalysis A: General*, *488*, 37-47.

McCracken, C., Dutta, P. K., & Waldman, W. J. (2016). Critical assessment of toxicological effects of ingested nanoparticles. Environmental Science: Nano, 3(2), 256-282.

Nations, U. (2015). Department of Economic and Social Affairs. Population Division.

Naylor, R.L., Hardy, R.W., Buschmann, A.H., Bush, S.R., Cao L., Klinger, D.H., Little, D.C., Lubchenco, J., Shumway, S.E., & Troell, M. (2021). A 20-year retrospective review of global aquaculture. Nature, 591,251-263. https://doi.org/10.1038/s41586-021-03308-6.

Patel, J., Sharma, D., Yadav, S., Lemtur, A., & Suman, P. (2019). Performance improvement of nano wire TFET by hetero-dielectric and hetero-material: At device and circuit level. *Microelectronics Journal*, *85*, 72-82.

Rosales-Mendoza, S., & González-Ortega, O. (2019). Nanovaccines. Springer International Publishing.

Sarkar, B., Mahanty, A., Gupta, S. K., Choudhury, A. R., Daware, A., & Bhattacharjee, S. (2022). Nanotechnology: A next-generation tool for sustainable aquaculture. Aquaculture, 546, 737330.

Shaalan, M., Saleh, M., El-Mahdy, M., & El-Matbouli, M. (2016). Recent progress in applications of nanoparticles in fish medicine: a review. Nanomedicine: Nanotechnology, Biology and Medicine, 12(3), 701-710.

Shah, B. R., & Mraz, J. (2020). Advances in nanotechnology for sustainable aquaculture and fisheries. Reviews in Aquaculture, 12(2), 925-942.

Thompson, K. D., Rodkhum, C., Bunnoy, A., Thangsunan, P., Kitiyodom, S., Sukkarun, P., ... & Pirarat, N. (2023). Addressing nanovaccine strategies for tilapia. Vaccines, 11(8), 1356.

Tokgun, O., Demiray, A., Kaya, B., Karagür, E. R., Demir, E., Burunkaya, E., & Akça, H. (2015). Silica nanoparticles can induce apoptosis via dead receptor and caspase 8 pathway on A549 cells. Advances in Food Sciences, 37(2), 65–70.

Turan, F., & Ergenler, A. (2023). The Genotoxic Damage in Cyprinus carpio Exposed to Abamectin. *Natural and Engineering Sciences*, *8*(2), 119-128.

TÜYLEK, Z. (2017). Nanoteknolojinin çevre ve insan sağlığı üzerindeki riskleri. *Kilis 7 Aralık Üniversitesi Fen ve Mühendislik Dergisi*, *2*(2), 1-12.

Vales, G., Demir, E., Kaya, B., Creus, A., & Marcos, R. (2013). Genotoxicity of cobalt nanoparticles and ions in Drosophila. Nanotoxicology, 7(4), 462–468. https://doi.org/10.3109/17435390.2012.689882

Vartak, A., & Sucheck, S. J. (2016). Recent advances in subunit vaccine carriers. Vaccines, 4(2), 12.Han, X., Xu, K., Taratula, O., & Farsad, K. (2019). Applications of nanoparticles in biomedical imaging. *Nanoscale*, *11*(3), 799-819.

Wu, L. P., Ficker, M., Christensen, J. B., Trohopoulos, P. N., & Moghimi, S. M. (2015). Dendrimers in medicine: therapeutic concepts and pharmaceutical challenges. *Bioconjugate chemistry*, *26*(7), 1198-1211.

Xia, T., Kovochich, M., Liong, M., Madler, L., Gilbert, B., Shi, H., ... & Nel, A. E. (2008). Comparison of the mechanism of toxicity of zinc oxide and cerium oxide nanoparticles based on dissolution and oxidative stress properties. ACS nano, 2(10), 2121-2134.

Yang, Z., Yue, G. H., & Wong, S. M. (2022). VNN disease and status of breeding for resistance to NNV in aquaculture. *Aquaculture and Fisheries*, *7*(2), 147-157.

Yochabedh, C. A., Nandhini, L., Preetha, R., & Rejish Kumar, V. J. (2023). Nanomaterials in aquatic products and aquatic systems, and its safety aspects. Applied Nanoscience, 1-14.

Zhang, H., Ji, Z., Xia, T., Meng, H., Low-Kam, C., Liu, R., ... & Nel, A. E. (2012). Use of metal oxide nanoparticle band gap to develop a predictive paradigm for oxidative stress and acute pulmonary inflammation. *ACS nano*, *6*(5), 4349-4368.

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