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PREFACE

The phytoestrogenic compounds in lavender may have an impact on hormonal balance. This book explains that the effects of phytoestrogens on reproductive physiology can differ based on a number of variables, including dosage, compound profile, and application technique.

Coccinellids harbor a rich and diverse gut microbiome, showing symbiotic relationships with microbes, which influence their survival and reproductive fitness. Recent scientific sources are reviewed on the gut microbiota of the coccinellids to understand the functional diversity of the microbiome inhabiting the gut of predaceous coccinellids.

This book unveiles that *Phanerochaete chrysosporium* can be used as a model organism in the evaluation of the toxic effect of cerium.

In addition, it is determined that *Daphnia magna* can be used in the evaluation of toxicity of zinc and zinc oxide nanoparticles in aquatic system by monitoring metallothionein biomarker.

The topics covered in this book are presented to the readers as a fundamental resource containing current information in the field of biological science.

Editor

Prof. Dr. Ali BİLGİLİ

Content

CHAPTER I

Does Lavender Herbal Tea (*Lavandula angustifolia* L.) Affect the Estrous Cycle?

Emine İnci BALKAN[1]
Bülent GÜNDÜZ[2]

Introduction

The Lamiaceae family includes the lavender plant (*Lavandula angustifolia* L.), which has been used for centuries for medicinal purposes and is particularly well-known for its aromatic essential oils. In addition, it is often preferred in various cultures due to its relaxing, sedative and antidepressant effects. In addition, it is often preferred in various cultures due to its relaxing, sedative and antidepressant effects (Cavanagh & Wilkinson, 2002). Lavender has been studied recently for its possible effects on the reproductive and endocrine systems in addition to its effects on the nervous system (Lis-Balchin & Hart, 1999; Henley & et al., 2007). In this regard, lavender's phytoestrogenic features are of interest, particularly in light of their implications for women's reproductive health

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(Tisserand & Young, 2013). The fact that lavender tea also carries this potential is in line with the increasing popularity of plant-based treatment approaches.

The female reproductive cycle in mammals, particularly in rodents, is known as the estrous cycle, and it is controlled by the interaction of key hormones such as estrogen, progesterone, follicle stimulating hormone (FSH), and luteinizing hormone (LH). The integrity of the hypothalamic-pituitary-ovarian axis and the reproductive health of the female reproductive system can be determined by evaluating the estrous cycle in experimental animals. The effects of medicinal products and chemicals on reproductive function can also be examined. These effects are often manifested as changes in the normal morphology, cytology, and histology of reproductive organs, as well as changes in the length of specific estrous cycle phases. The female reproductive system depends on the estrous cycle phases to remain functional, and these phases are extremely sensitive to diet, stress, and environmental factors (Hawkins & Matzuk, 2008). The estrous cycle includes four phases: proestrus, estrus, metestrus, and diestrus, lasting 4 to 5 days.

Phytoestrogenic compounds are natural compounds found in plants that exhibit estrogen-like activities, and these compounds are known to alter hormonal regulation by interacting with estrogen receptors in mammals (Nikolić & et al. 2017). These phytoestrogenic compounds found in lavender have the ability to specifically alter hormone levels, which in turn can control the different stages of the estrous cycle (Zava & et al., 1997).

Research on the phytoestrogenic properties of lavender tea has shown that even at low concentrations, lavender may have an estrogenic effect (Henley & et al., 2007). Studies on the relationship between lavender essential oil and estrogen receptors, however, indicate that this relationship is weak and that lavender works best at specific dosages (Ramsey & et al., 2019). Given the stress-reducing properties of lavender, it is thought that these effects may be particularly useful in alleviating stress-induced reproductive disorders (Gorgini & et al., 2021). Lavender tea has the potential to

positively impact the hypothalamus-pituitary-gonadal (HPG) axis by mitigating the effects of stress, which can disrupt reproductive processes (Umezu & et al., 2002).

Phases of the estrous cycle may change as a result of interactions between hormone levels and the estrogenic activities of phytoestrogens, particularly lavender (Zava & et al., 1997). Understanding these potential effects of lavender is of great importance in increasing the reliability of herbal treatments and establishing a solid scientific basis for their use. However, the current literature on how lavender tea may affect the estrous cycle is limited, which necessitates further research on the subject.

A crucial first step in increasing the potential applications of natural remedies and guaranteeing their safety is to comprehend how lavender tea and related herbal products affect the endocrine system. The purpose of this section is to provide a foundation for addressing the gap in the literature by thoroughly examining the potential impacts of lavender tea on the estrous cycle.

Material and Method

Animals

In the study, 6-week-old female Syrian hamsters (*Mesocricetus auratus*) weighing approximately 55-60 g were used. 20 female hamsters were randomly divided into 4 groups, each group consisting of 5 hamsters. The hamsters were obtained from the colony in the Hamster and Gerbil unit of Çanakkale Onsekiz Mart University, Experimental Animals Research and Application Center (COMUDAM). During the experiment (12 days), the laboratory temperature was set at approximately 22 ± 2 ºC. Hamsters were kept individually in polycarbonate cages (16x31x42 cm). The photoperiod was set at 16 hours of light and 8 hours of darkness. Hamsters were given food pellets and tap water ad libitum. All methods were performed with the permission of Çanakkale Onsekiz Mart University, Experimental Animals Ethics Committee, decision number 2021/04-10.

Experimental design

The study was conducted in four groups. The first group (n=5) was given only tap water as a control group. The treatment groups were given lavender tea (50 mg/kg, 100 mg/kg and 200 mg/kg) added to tap water.

Preparation of lavender tea

Dried flowers of lavender plant were obtained from a local herbalist in Çanakkale. Thirty grams of lavender plant was tead twice at 80 ^{0}C with 200 mL of water for 4 hours and filtered. Water was evaporated under vacuum (90-100 ^{0}C). The residue was then dissolved in physiological serum to obtain a final concentration of 100 mg/mL.

Application of lavender tea to animals

The control group was given tap water. Lavender plant tea (6 g) were added to the animals' drinking water for oral administration in the experimental groups (50 mg/kg, 100 mg/kg, and 200 mg/kg). Every day, the water in the bottles was replaced. Female animals were brought back to the colony at the conclusion of the experiment (the 12th day), and their recovery was tracked.

Vaginal smear application

Vaginal smear samples were taken from the animals to determine the phases of the estrous cycles. Each smear application was examined individually under a light microscope. When taking a vaginal smear, approximately 0.5 ml of physiological serum was drawn into the pipette. The animal was held with its belly facing up and the pipette tip was placed into the vagina. The physiological serum in the pipette was given inside and the liquid given was withdrawn with the pipette again without removing the pipette tip from the vagina. The liquid drawn with the pipette was placed on a cleaned slide and the slide was left to dry. After drying, the slides were placed in the prepared methylene blue chalices and kept in the

dye for 30 minutes. At the end of the period, the slides were removed from the dye and washed with pure water. After the washed slide samples dried, they were examined under a light microscope to determine the estrous cycle phases (Nishino & Totsukawa, 1996). The estrous cycle was monitored and recorded by daily examination of the vaginal mucosa to determine the day the epithelium ruptured. The day after ovulation was considered day 1 of the estrous cycle.

Results

Proestrus phase

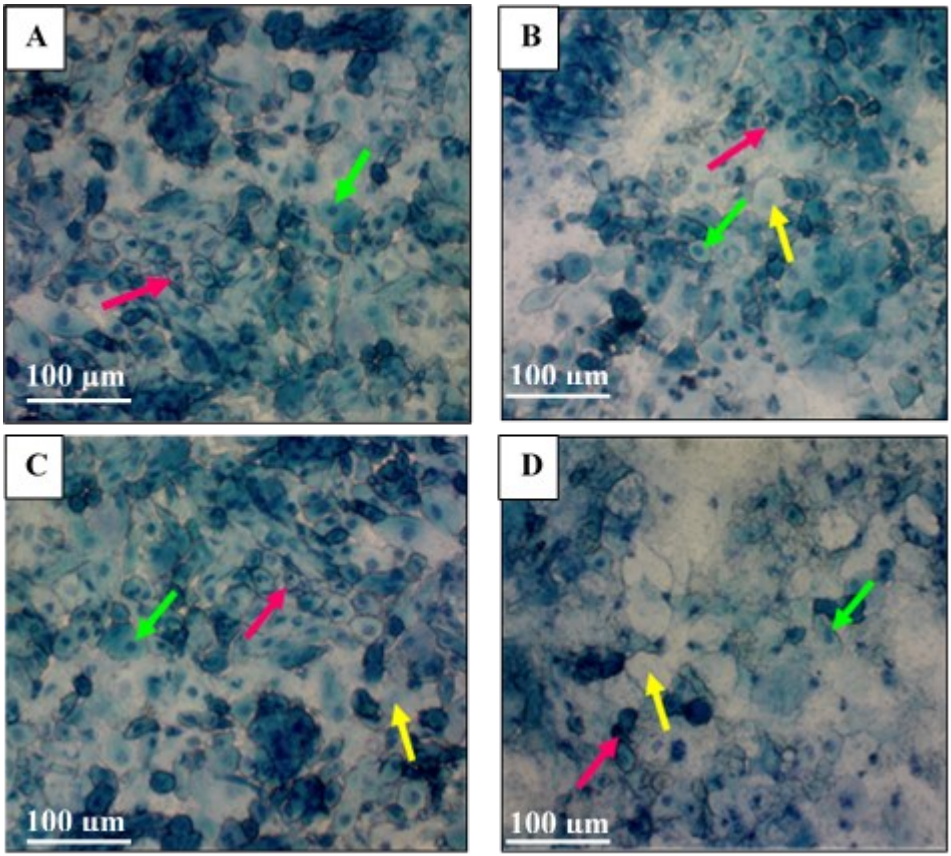


Figure 1: Cells in the proestrus phase (shown with arrow) in the control group (A), 50 mg/kg (B), 100 mg/kg (C) and 200 mg/kg (D) experimental groups.

→ Small nucleated interstitial epithelial cell

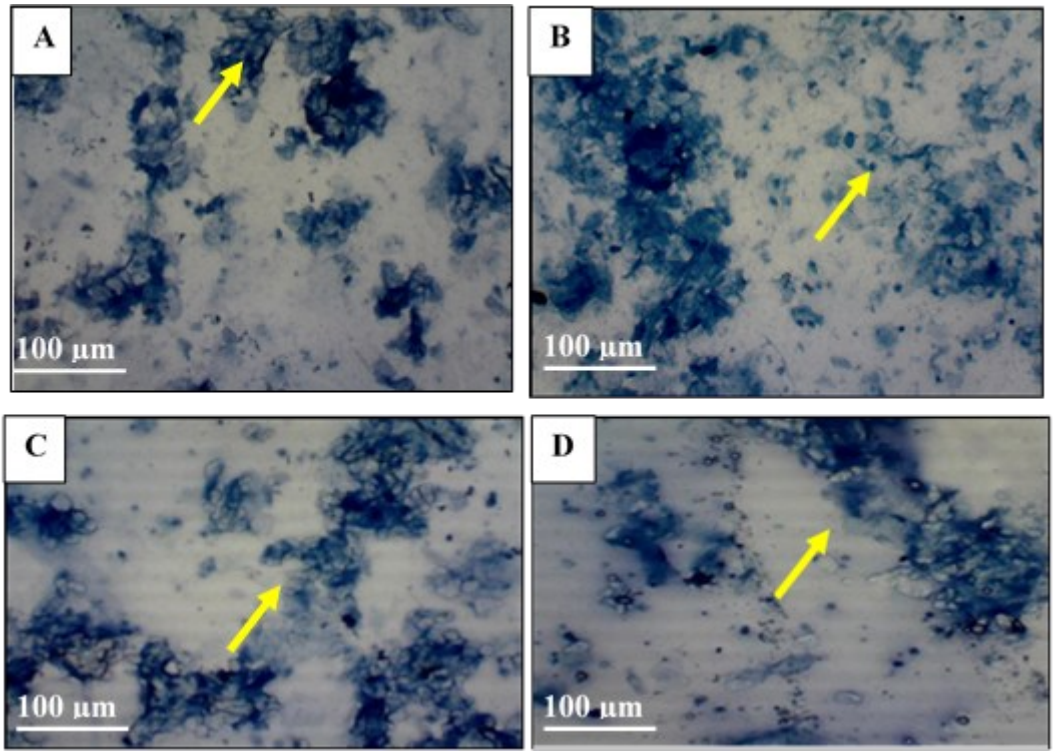
→ Large nucleated interstitial epithelial cell

→ Anucleated cornified cell with irregular edges

An increase in the number of cornified cells with anucleated irregular edges was observed in the group to which lavender extract was added at a dose of 200 mg/kg to tap water.

Estrus phase

Figure 2: Cells in the estrus phase (shown with arrow) in the



control group (A), 50 mg/kg (B), 100 mg/kg (C) and 200 mg/kg (D) experimental groups.

→ Anucleated cornified cell with irregular edges

An increase in the number of cornified cells with anucleated irregular edges was observed in the second (group with 100 mg/kg lavender extract added to tap water) and third groups (group with 200 mg/kg lavender extract added to tap water).

Metaestrus phase

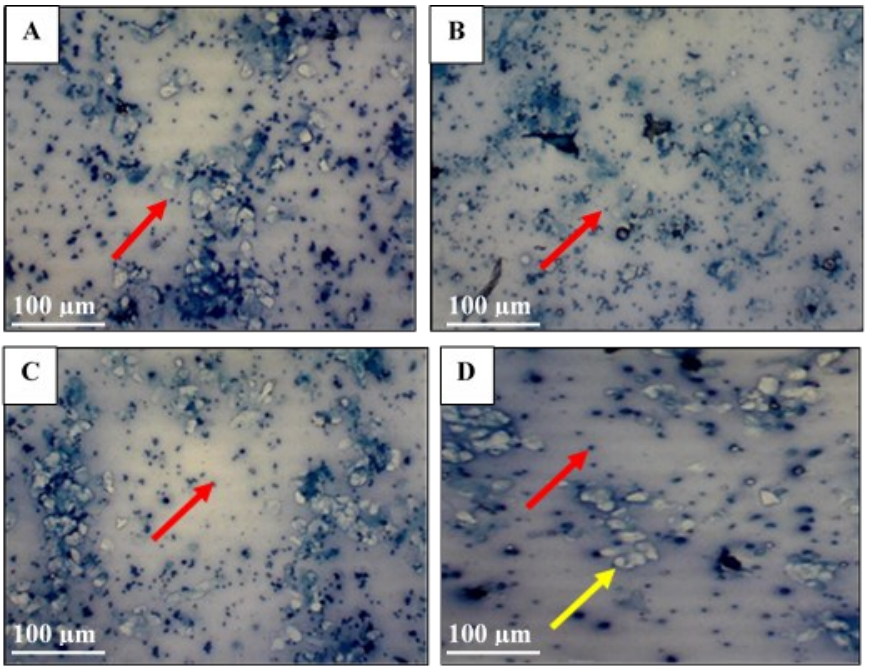


Figure 3: Cells in the metaestrus phase (shown with arrow) in the control group (A), 50 mg/kg (B), 100 mg/kg (C) and 200 mg/kg (D) experimental groups.

Leukocyte

Anucleated cornified cell with irregular edges

In the first group (the group to which 50 mg/kg of lavender extract was added to tap water), an increased number of leukocytes was observed. In the third group (the group to which 200 mg/kg of lavender extract was added to tap water), an increase in the number of leukocytes and anucleated irregularly-edged cornified cells was observed.

Diestrus phase

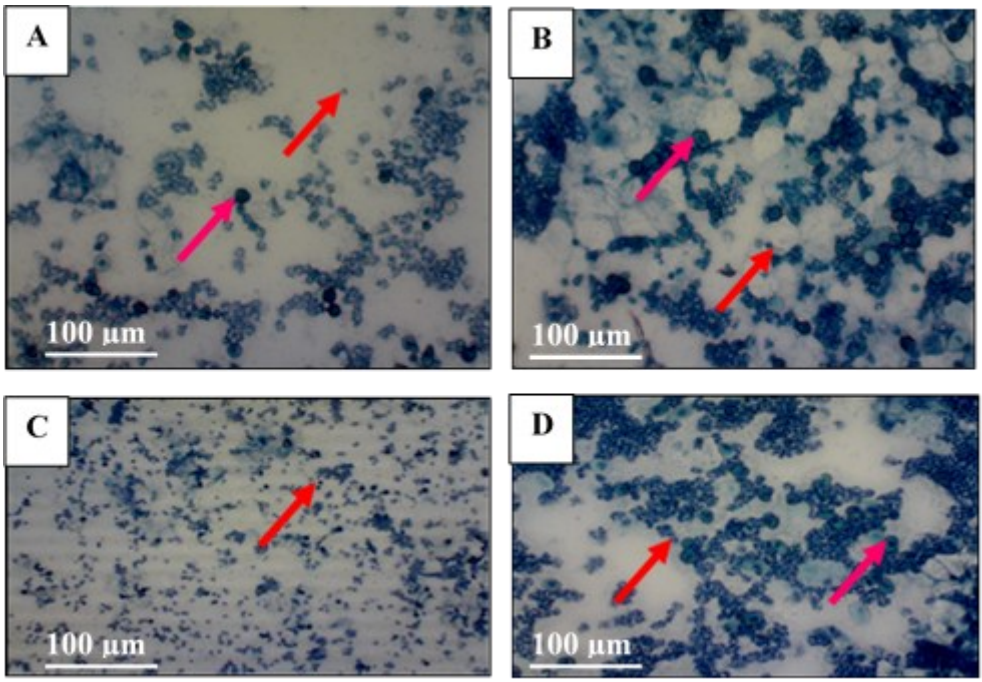


Figure 4: Cells in the diestrus phase (shown with arrow) in the control group (A), 50 mg/kg (B), 100 mg/kg (C) and 200 mg/kg (D) experimental groups.

→ Leukocyte

→ Small nucleated interstitial epithelial cell

In the second group (the group to which 100 mg/kg lavender extract was added to tap water), an increase in the number of leukocytes was observed, while in the first group (the group to which 50 mg/kg lavender extract was added to tap water) and the third groups (the group to which 200 mg/kg lavender extract was added to tap water), an increase in the number of small nucleated interstitial epithelial cells was observed.

Discussion and Conclusion

The genital organs of female mammals undergo a number of physiological and histological changes throughout the estrous cycle. The vaginal smear method can be used to determine the estrous stages by examining these changes (Cora & et al., 2015). This method was used in our study to identify the stages of the estrous cycle in Syrian hamsters. The control group's estrous cycle lasted roughly four days, which is in line with prior studies.

Significant differences existed in the effects of varying lavender tea doses on the estrous cycle. The proestrus, estrus, and metaestrus phases showed an increase in the number of cornified cells with irregular edges, while the diestrus phase showed an increase in small nucleated interstitial epithelial cells, particularly in the 200 mg/kg dose group. These results suggest that the phytoestrogenic compounds in lavender may have an impact on hormonal balance (Zava & et al., 1997; Henley & et al., 2007). It is possible that lavender's effects on the estrus cycle could have a wider impact on the brain and reproductive system. The cycle between gonadotropin-releasing hormone (GnRH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), and estrogen is what regulates the estrus cycle, so changes in the levels of these hormones may also have an impact on the estrus cycle.

The effectiveness of such herbal substances can be increased by consuming them with nutrients, even though they can be taken as an extract alone. Studies by Morin (1986) and Dickerman & et al. (1993), for instance, emphasize how environmental and nutritional factors affect female hamsters' estrous cycles. Research has demonstrated that restricting nutrition suppresses ovulation and estrous behaviors, but normalizing nutrition causes these behaviors to resurface.

Human studies have demonstrated that, even at low concentrations, the phytoestrogenic qualities of lavender essential oil can adversely affect the human reproductive system through the endocrine pathway. Henley and his colleagues (2007) demonstrated,

for instance, that exposure to tea tree and lavender oils was associated with prepubertal gynecomastia, or abnormal breast development in males. Based on these results, the team found that premature thelarche, a condition linked to exposure to lavender-scented products, was also present. This condition causes early breast development in girls under the age of eight, when there are no other signs of puberty. To determine the underlying mechanism, Henley and his team investigated the effects of eight compounds found in lavender and tea tree oils. Some of the compounds had varying degrees of estrogenic or anti-androgenic properties, as evidenced by their effects on estrogen receptor alpha and androgen receptor activity in human cells. The findings indicated that compounds found in essential oils may promote breast growth by increasing estrogen activity while inhibiting androgen activity. It was concluded that repeated topical exposure to lavender and tea tree oils, particularly in boys, most likely causes prepubertal gynecomastia. In our study, a high dose of 200 mg/kg had a significant effect on the estrus cycle, while lower doses showed only minor changes. This highlights the dose-response relationship of phytoestrogens (Ramsey & et al., 2019).

The olive leaf is another significant plant that is widely utilized in society. A thorough investigation of the impact of olive leaf extract on hamsters' estrous cycles was conducted by Özsalmanlı in 2022. Different doses of olive leaf extract (20, 40, and 80 mg/kg) had effects on hormones and histology, according to Özsalmanlı's findings. The results of Özsalmanlı's study confirmed ours, showing that phytoestrogenic effects can alter hormonal balance and increase the number of cornified cells. Nonetheless, given that olive leaf extract works at lower dosages, it would seem that the bioactive compound profiles of the two herbal extracts differ.

Lavender oil is one of many herbal teas or extracts that have not yet been thoroughly studied but may have health risks. These herbal ingredients should be further studied, particularly since they

can be purchased without a written prescription from a doctor and should be used carefully.

In conclusion, the results of our study and other research indicate that the effects of phytoestrogens on reproductive physiology can differ based on a number of variables, including dosage, compound profile, and application technique. When paired with their ability to reduce stress, lavender extract's estrogenic effects—especially when taken in high doses—support their potential role in reproductive health. It is important to look beyond lavender tea's phytoestrogenic qualities in order to comprehend how it affects reproductive health. It ought to look at the interplay between lavender's stress-relieving and estrogenic properties, particularly how it controls hormonal balance and how the estrous cycle reflects this control. Further research on the lavender plant will make it easier to see how herbal treatments may improve reproductive and endocrine health.

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CHAPTER II

Gut Microbiome in Predaceous Coccinellids

Meena YADAV
Ahmad PERVEZ
Hakan BOZDOĞAN

1.Introduction

Insects constitute one of the most diverse and abundant life forms on earth. The gut of the insects is colonized by myriad forms of microbiota, which help the insects survive in changing environmental conditions. Insects effectively depend on their gut microbiota for normal physiological functions and survival. The gut microbiota includes all forms of microbes available in the digestive tract such as bacteria, fungi, viruses, protozoa, and archaea. These microbes have a complex relationship with the host affecting their metabolism and other physiological functions (Nikolouli et al., 2022). There are many benefits of this symbiotic association: 1. Microbes synthesize certain nutrients that are lacking in the natural

diets of the host; 2. Secrete enzymes to digest food; 3. Improve host immunity, and resist predation by natural enemies or pathogenic bacteria; 4. Impact the development, reproductive attributes, and the hosts' life history (Xie et al., 2024). The gut microbiota is passed on from one generation to the next in the host via vertical inheritance (Hammer & Moran, 2019). However, this passage of microbiota depends on the insects' diets, particularly the diets of predaceous coccinellids (Wang et al., 2024). These coccinellids are predaceous on numerous phytophagous insect pests, viz. aphids, scale insects, mealybugs, whiteflies, thrips, etc., which induce severe crop damage and yield loss (Omkar and Pervez, 2016; Pervez et al., 2020). The gut microbiome in coccinellids largely consists of symbiotic bacteria, which have digestive and protective functions in the ladybirds. However, numerous bacteria inculcate detrimental effects on the ecology of the ladybirds, like compromising the fitness components of the ladybirds, disturbing their sex ratio by killing male embryos, etc. In this chapter, we reviewed recent literature on the gut microbiota of the coccinellids to understand the functional diversity of the microbiome inhabiting the gut of predaceous coccinellids.

2.GUT MICROBIOTA IN COCCINELLIDS

A diverse range of gut symbionts in insects help them in adapting to extreme and diverse environments, and also in expanding their niches. This makes them to reach every niche on the earth (Rupawate et al., 2023). The survival of coccinellids is directly or indirectly dependent on the number and diversity of the gut symbionts, which depends on several factors, including varied diet (Pervez and Yadav, 2018). Coccinellids are also dependent on their

gut microbiota for successful survival viz. reproduction, physiology, metabolism, and detoxification (Du et al., 2022a). The most common bacterial phyla observed in ladybirds include Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Thermotogae and Cyanobacteria (Dudek et al., 2017).

Harmonia axyridis (Pallas) is a generalist ladybird and a potential predator, with a wide range of prey such as aphids, scabies, mealybugs, scale insects, *etc*. It has been widely used in biological control programmes with satisfactory results. A common observation is that the gut microbiota diversity depends largely on the food sources (Huang et al., 2022). The gut microbiota abundance increased when *H. axyridis* ate conspecific eggs and aphids, while the same decreased when it ate *Coccinella septempunctata* L. eggs and aphids (Wang et al., 2022). The feeding by the beetle on native and non-native prey changes its gut microbiota, however, this change reverses slowly and diminishes after a few generations, suggesting that the beetle has its core gut microbiota, which is instrumental in its adaptation and reproductive success (Wang et al., 2024).

The variations in the gut microbiota in the North American and Asian populations of *H. axyridis* suggest that this microbial community is largely restructured in various zoogeographical populations and depends on the diets they are conditioned to take (Li et al., 2022). Likewise, the gut microbiota is restructured when the adults and larval stages undergo cannibalism and intraguild predation (Wang et al., 2022). Adults *H. axyridis* can harbor richer and more diverse gut microbiota when fed on non-native prey than native prey species (Gao et al., 2022). The adult *H. axyridis*

inhabiting the non-native range had poorer microbiota richness and evenness than the ones in the native range (Li et al., 2022).

The diversity of gut microbiota changes with the developmental stages of ladybirds. For example, in *H. axyridis*, higher bacterial richness and diversity are found in eggs while pupae show least diversity and richness among the three stages *viz*. eggs, pupae and adults. The dominant bacterial phyla in *H. axyridis* are Proteobacteria, Actinobacteria and Firmicutes. The pupae show more presence of Firmicutes, while adults harbor Proteobacteria predominantly (Du et al., 2022a).

Serratia symbiotica is a toxic bacterial symbiont living in various prey species and inhibits the survival of their natural enemies (Perreau et al., 2021; Renoz et al., 2021). However, in ladybirds, its negative effects are not found and contrarily it provides nutritional benefits to predatory ladybirds like *Micraspis discolor* and other members of the tribe Coccinellini (Du et al., 2022b). There are many more microbes in the guts of ladybirds that exist in symbiotic relationship with them, and help in digestion and absorption of food (Refer Table -1).

Table 1: Symbiotic Microbes in Coccinellids

S. No.	Coccinellids	Major microbes in the Coccinellid gut	References
1	*Coccinella septempunctata* L.	Bacteria: Proteobacteria is the dominant Phylum, followed by Bacteroidota and Actinobacteriota; major genera include *Rhodobacter, Methylovigula, Burkholderia, Bradyrhizobium*	Lu et al. (2024)
2	*Coccinella transversoguttata*	32 bacterial species belonging to Proteobacteria	Zhang & Yang (2024)
3	*Harmonia axyridis* (Pallas)	Bacteria: *Serratia*, *Enterococcus* and *Enterobacter, Hafnia-Obesumbacterium, Enterobacteriaceae, Staphylococcaceae, Streptococcaceae, Micrococcaceae* Fungi: *Davidiellaceae, Wallemiaceae, Incertae_sedis,*	Huang et al. (2022); Luo et al. (2023)
		Bacteria: *Enterobacter, Klebsiella, Enterobacteriaceae*_unclassified, *Enterobacterales*_unclassified, and *Serratia*	Xie et al. (2024)
		Romboutsia, *Escherichia-Shigella*, *Bacteroides*, *Terrisporobacter*, *Enterobacter*, *Lactobacillus*, *Fusobacterium*, *Actinobacillus*, *Sphaerochaeta*,	Hu et al. (2022)
		Enterococcus, *Serratia* and *Enterobacter*	Luo et al. (2023)

		Bacteria: *Spiroplasma* and *Wolbachia*	Awad et al. (2023)
		Staphylococcus, Acinetobacter, Enterobacter, Serratia and *Glutamicibacter*	Du et al. (2022a)
4	*Henosepilachna vigintioctopunctata* (Fabricius)	*Acinetobacter soli* and *Acinetobacter ursingii* (Acinetobacter, Moraxellaceae); *Moraxella osloensis* (Enhydrobacter, Moraxellaceae); and *Empedobacter brevis* (Empedobacter, Weeksellaceae).	Li et al. (2021b)
5	*Novius pumilus*	*Serratia, Pseudomonas, Lactococcus*	Tang et al. (2024)
6	*Propylea japonica* Thunberg	*Staphylococcus*, *Enterococcus,* and *Acinetobacter*	Zhang et al. (2019)

3.FACTORS INFLUENCING GUT MICROBIOTA DIVERSITY IN COCCINELLIDS

3.1. Diet

The diversity in the prey quality increases the microbiome richness in the gut of predaceous ladybirds (Tiede et al., 2017). The diversity of gut microbiota changed in *Henosepilachna vigintioctopunctata* (Fabricius), a coleopteran when fed on two types of diets, *viz*. two host plants (*Solanum nigrum* and *Solanum melongena*) and an artificial diet. The beetles that fed on *S. nigrum* showed a richer diversity of gut microbes than the other two diets. The microbes are involved in lipid metabolism, which affects insect fecundity. In the *S. nigrum* diet, enhanced development of testes and ovarioles was observed, thereby impacting their reproductive output

(Li et al., 2021). Natural diets provide more support for survival and abundance of gut microbiota in coccinellids, which in turn increases the fecundity of the latter, compared to artificial diets (Huang et al 2022). However, artificial diets may be manipulated to elevate the gut microbiota richness, i.e., synergistic probiotics (eg. glucose and trehalose) may be identified in artificial diets. The major microbiome, in *H. axyridis* that were reared on aphids, includes Proteobacteria and Firmicutes, which changed in those fed on artificial diets (Xie et al., 2024).

3.2. Allelochemicals and Plant Secondary Metabolites

Allelochemicals and plant secondary metabolites induce both positive and negative roles in the microbiome richness and the fitness components of predaceous ladybirds. The secondary metabolite, azadirachtin found in Neem damages midgut epithelial cells, a center for digestion and detoxification in *H. axyridis*. It increases the abundance of bacterial species, *viz. Serratia*, *Enterococcus,* and *Enterobacter*, but decreases the abundance of *Hafnia-Obesumbacterium*, and this observation coincides with the inhibition of growth and development of the beetle, suggesting the negative impact of azadirachtin treatment on *H. axyridis* (Luo et al 2023). Similarly, glyphosate exposure disturbs the microbiota of ladybirds, like *H. axyridis,* and reduces their body weights (Gao et al., 2023).

The levels of volatile alkyl pyrazines, *viz.* methyl- and methoxy pyrazines found in reflex bleeds of ladybirds, like *H. axyridis* are associated with intracommunication, and attractant functions increased with the gut-bacteria, *Serratia* and *Lactococcus* (Schmidtberg et al., 2019).

3.3. Herbicides and Pesticides

Herbicides and pesticides show non-target effects on the organisms, including ladybirds, associated with agro-ecosystem. For example, when ladybirds consume herbicides and pesticides present on pollen and/or prey, their survival rate reduces and fecundity increases. These changes are associated with relative changes in the diversity of microbiota such as Serratia, *Staphylococcus*, Enterobacter, and *Hafnia-Obesumbacteriu* (Gao et al., 2023).

3.4. Horizontal Transfer

Bacteria can show two types of transfer between ladybirds and their prey, viz., vertical transfer and horizontal transfer. Vertical transfer occurs between different developmental stages of ladybirds (Gao et al., 2023), while horizontal transfer occurs between prey and predaceous ladybirds, both of which act as hosts for the microbiota. For example, *Serratia symbiotica*, a common bacterium in the aphids, was transferred from *M. japonica* to *Propylea japonica*, *C. septempunctata*, and *Cryptolaemus montrouzieri* via predation. However, the ladybirds can also transfer the bacteria back to aphids via their droppings, suggesting that these bacteria survive in their digestive tracts. By doing this, bacteria are able to expand their niches (Du et al., 2023).

3.5. Cold Storage

Cold storage is often used to increase shelf life of insects that are used as a biological control tool. However, the cold treatment shows detrimental effects as well, such as a significant decrease in egg hatchability of ladybirds viz. *H. axyridis*, during oviposition later on. This might be due to changes in the bacterial microbiome

composition in cold-stored *H. axyridis* (Sun et al., 2024). However, the bacteria and Coccinellids show intricate relationships, which are yet to be explored.

4.HARMFUL MICROBES ASSOCIATED WITH COCCINELLIDS

Male-killing bacteria, commonly known as male killers, are microbiota that kill male embryos of ladybirds. These reproductive manipulators are common maternally-inherited male killers, *viz*. *Wolbachia, Rickettsia,* and *Spiroplasma*, which have been largely responsible for high female-biased sex ratios in a ladybird, *Adalia bipunctata* L. (Archer et al., 2023). The male killers come from diverse microbiome groups, which include flavobacteria, ɣ-proteobacteria, α-proteobacteria, and Mollicutes (Majerus, 2006). In the U.K. and most European nations, *A. bipunctata* is a common victim of these male killers, which are found in high frequencies in its populations (Archer et al., 2023).

Parasitism in coccinellids is sometimes enhanced due to endosymbionts. For example, in *H. axyridis* having endosymbiont bacterium *Spiroplasma*, co-infection by the ectoparasitic fungus *Hesperomyces harmoniae*, neither protected the host nor compromised its health. However, it resulted in low fecundity and a low hatchability rate (Awad et al., 2023). However, the gut biome structure is altered if predaceous ladybirds are parasitized. For instance, overall 58.5% of bacterial OTUs (Operational Taxonomic Units) were changed in a seven-spotted ladybird, *Coccinella septempunctata* L. after being parasitized by a parasitoid wasp, *Homalotylus eytelweinii* (Wang et al., 2023). Proteobacteria abundantly increased in the parasitized ladybirds, while the

abundance of Firmicutes decreased compared to the unparasitized ones. Likewise, the percentage of *Aeribacillus* decreased significantly in various life stages of the parasitized ladybirds compared to the unparasitized ones. Parasitism in the early instars of ladybirds may also lead to the rise in the α-diversity of the gut microbiome. Similar alterations were also found in the β-diversity of the gut microbiota in the parasitized ladybirds (Wang et al., 2023).

Entomopathogenic nematodes of the genera *Steinernema* and *Heterorhabditis* may harbor numerous bacteria, which become virulent after entering the gut of the host ladybirds (Ceryngier and Hodek, 1996). In addition, bacteria-induced parasitism can lead to sex-specific modulations in the predaceous ladybirds, as the parasitic nematode *Parasitylenchus bifurcatus* can alter the sex ratios of *H. axyridis* (Gegner et al., 2018). A few nematode-associated bacteria, such as *Serratia marcescens* and *Providencia rettgeri* are virulent in *H. axyridis* and induce sex-specific changes by suppressing the immunity-related genes associated with the antimicrobial peptides. However, the female immune system against this nematode-coupled microbial attack is much stronger than the male ones. Some microbes that are harmful to coccinellids have been summarised in Table 2.

Table 2: *Harmful Microbes Associated with Coccınellids*

S. No.	Coccinellids	Harmful Microbes	References
1	*Harmonia axyridis* (Pallas)	*Hesperomyces harmoniae* (Ascomycota: Laboulbeniales); Spiroplasma	Awad et al 2023
		Burkholderia, *Rhodococcus*, *Chlamydiae* and *Anaplasmataceae* spp. (*Neorickettsia helminthoeca* and *Ehrlichia ovina*), *Wolbachia pipientis*, *Spiroplasma* spp., *Wolbachia* spp. and *Rickettsia* spp. (Pathegens)	Dudek et al., 2017
		Serratia symbiotica, and *Regiella insecticola* (Reduce fitness)	Kovacs et al. (2017)
		Wolbachia, Spiroplasma, (Male killers)	Li et al. (2021a)
2.	*Hippodamia convergens* Guérin-Méneville	*Hamiltonella defensa* and *Serratia symbiotica* (Reduce fitness)	Costopoulos et al. (2014)
		Wolbachia, Spiroplasma, and *Rickettsia* (Male killers)	Kovacs et al. (2017)
3	*Adalia bipunctata*	*Spiroplasma*	Archer et al. (2023)

5.CONCLUSION

Coccinellids harbor a rich and diverse gut microbiome, showing symbiotic relationships with microbes, which influence

their survival and reproductive fitness. However, some of the microbes are harmful too for the ladybirds. Both these associations need to be understood explicitly. In the augmentation programmes, a healthy population of ladybirds can be reared by maintaining a healthy gut microbiome by feeding them on suitable diets. The gut microbe diversity changes with parasitism in ladybirds, opening new avenues for role of microbes in host-parasitoid interactions. However, the studies addressing these aspects are lacking, hence, more future studies are necessary to better understand the role of gut microbiome in survival and interactions of ladybirds with other organisms.

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CHAPTER III

White rot fungus *Phanerochaete chrysosporium* as an in vitro model to investigate the potential toxicity of rare earth element cerium

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1. Introduction

The most abundant rare earth element is Ce, which is present at 64 ppm in the upper earth's crust (Zhang, 2016). CeO_2 and CeO_2 nanoparticles are widely used as components in polishing agents (for glass, television faceplates, mirrors, optical glass, silicon microprocessors and disk drives), catalytic converters, solid oxide fuel cells, sensors, fuel catalysts, and also is released into the atmosphere with diesel exhaust (Das et al., 2013; Snow et al., 2014).

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Because of their widespread use in different industries, Ce can be dispersed into the environment, causing putative changes in agriculture, aquatic fauna, and soil, which might, in turn, cause severe damage to human health (Gaiser et al., 2009). Reactive oxygen species (ROS) can be formed during electron transport (accumulation of reactive intermediates) in the mitochondrial membrane and can be formed by various mechanisms such as inactivation of antioxidant enzymes, depletion of non-enzymatic antioxidants, and membrane lipid peroxidation (Modesto and Martinez 2010). Oxidative stress is defined as an imbalance between the production of ROS during the oxidative metabolism in mitochondria and cellular antioxidant defenses (Betteridge, 2000). High exposure to ROS can cause elevated inflammatory effect (Sang et al., 2014), immune function damage (Cheng et al. 2014), oxidative stress damage (Ji et al., 2021), and reproductive toxicity (Li et al., 2021). In organisms, ROS react with lipids to cause peroxidation. TBARS has been widely used for many years as a convenient biomarker for lipid peroxidation (Ayala et al., 2014).

GSH is the first line of defense against oxidative stress. Therefore, the change observed in GSH levels can be a very important indicator of the detoxification ability of all living cells (Sun et al., 2006). GSTs are major phase II enzymes involved in metabolic detoxification processes. The task of GSTs is to form more soluble, non-toxic peptide derivatives and catalyze the conjugation of the tripeptide GSH with compounds containing an electrophilic center (carbon, nitrogen or sulfur) (Coleman et al., 1997). SOD, the first defense line against ROS, converts O_2 to H_2O_2. Subsequently, H_2O_2 is detoxified by CAT. The enzymatic action of

CAT leads to the formation of water and molecular oxygen (Qui et al., 2008).

P. chrysosporium is a white rot fungus has been widely used for treating heavy metal–contaminated agricultural waste, and wastewater containing heavy metals and toxic organic pollutants due to its strong ability to degrade persistent organic pollutants and remove heavy metals from wastewater or aqueous solutions (Iqbal and Edyvean, 2004; Sedighi et al., 2009).

In the present study, *P. chrysproium* was used as an in vitro model to study the potential toxicity of rare earth element Ce. To assess the toxicity of Ce; SOD, CAT, GST activities, and TBARS, GSH levels were analyzed in *P. Chrysosporium* exposed to different concentrations of Ce.

2. Materials and methods

2.1. Chemicals

Cerium chloride ($CeCl_3$) was purchased from Bostonchem USA. This chemical was used directly without further purification.

2.2. Fungus and pellet preparation

In present study, the white rot fungus *P. chrysosporium* (ME446) was used from the culture collection of Environmental Microbiology Laboratory in Munzur University. The stock *P. chrysosporium* culture in 2% sabouraud dextrose agar (SDA) solid medium in Petri dishes was passaged monthly for renewal purposes and kept in the refrigerator at +4°C until used in experimental studies. 2 plugs of 1 cm diameter were taken from this *P. chrysosporium* stock agar plate culture in sterile conditions in laminar flow and added to the medium containing 150 ml sabouraud

dextrose broth (SDB) in a 250 ml flask. This medium was placed in an incubator with orbital shaker with 150 rpm at 27 ºC. At the end of 7 days of incubation, wet pellets to be used for inoculation to be transferred to the application media were obtained.

2.3. Experimental Protocol

In the study, four different application groups were designed (Table 1). Experimental studies were carried out in 3 repetitions in each group.

Table 1. Application groups

Groups	Medium
X (Control)	Deionized water without $CeCl_3$ + 5 g wet pellets of *P. chrysosporium*
Y	0.5 mg/L $CeCl_3$ + 5 g wet pellet of *P. chrysosporium*
Z	1 mg/L $CeCl_3$ + 5 g wet pellet of *P. chrysosporium*
T	2 mg/L $CeCl_3$ + 5 g wet pellet of *P. chrysosporium*

The all application groups were sterilized by using JSR brand JSAT-80 model autoclave for 15 minutes at 121ºC. Before pellet inoculation, it was waited to cool down in Heal force brand HF-safe 1200 model laminar flow until it reached room temperature.

Each of these media cooled in laminar flow was prepared in 3 repetitions. 5 g of the previously prepared *P. chrysosporium* wet pellets were taken from the culture medium under sterile conditions and inoculated into X, Y, Z and T application media under sterile conditions. After inoculation, all media were placed in an orbital shaking refrigerated incubator operating at 150 rpm at 27ºC.

At the end of the 24th and 48th hours of the incubation, all the flask contents belonging to the application groups were filtered in the sterile cabinet and the Fungus pellets were taken and put into the falcon tubes. The samples were stored in a deep freezer at -20 ºC until the analysis process.

2.4. Homogenization of Pellets

To determine the antioxidant enzyme activities, 0.5 mg of wet pellet was taken from all groups at the end of the 24th and 48th hours of incubation was homogenized in 5-10 ml of cold buffer (0.1 M phosphate buffer (pH 7.0) 0.5 mM EDTA). Then, the supernatants were centrifuged at 10000 g for 15 min at 4ºC and stored at −80 °C until the enzyme activities were measured.

2.5. Biochemical analysis

In order to determine the biochemical response in present study, SOD, CAT, GST activities and GSH, TBARS levels were determined.

SOD, CAT, GSH and TBARS Assay kits used in the study were purchased from CAYMAN. Catalog numbers: (CAT: 707002, SOD: 706002, GSH: 703002, TBARS: 10009055). GST assay kits used in the study were purchased from Elabscience (cat.no: e-bc-k278-s).

2.6. Statistical analysis

Statistical analyzes were carried out using PASW Statistics 18 software. Duncan's multiple range test at $P<0.05$ level was used to determine the statistical differences among groups in the same application periods. The statistical differences between the

application periods (24 and 48 h) were determined by two-tailed independent-samples t test.

3. Results

In this study, SOD, CAT, GST activities and GSH and TBARS levels of *P. chrysosporium* exposed to different concentrations of Ce were determined.

3.1. SOD activity

SOD activity was statistically significantly decreased in all treatment groups at 24 and 48 hours compared to the control. ($p<0.05$). When the exposure times are compared; statistically significant differences were found between Z and T groups ($p< 0.05$) (Fig 1).

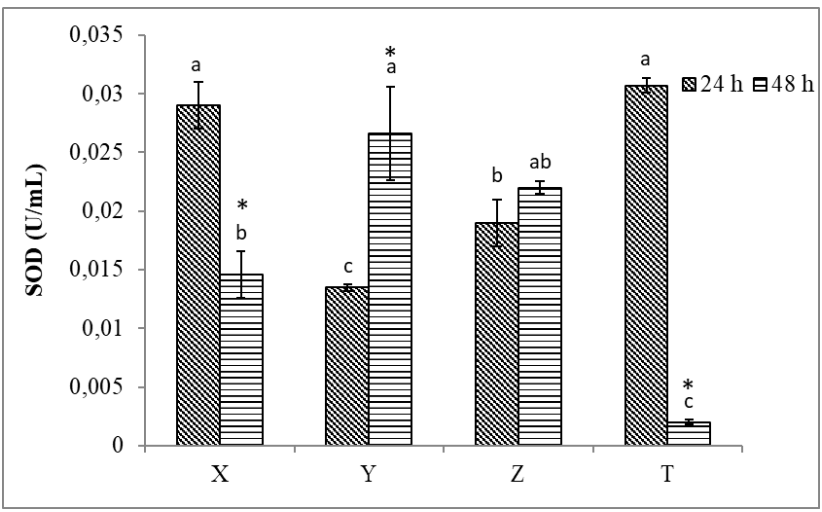

*Figure 1. Changes in SOD activity in P. chrysosporium exposed to rare earth element Ce. The different letters (a, b, c) show the statistical differences between different groups at the same time, and * show the statistical differences between different hours (24th and 48th hours) in the same group.*

3.2. CAT activity

CAT activities decreased significantly in all treatment groups after 24 and 48 hours compared to the control group ($p < 0.05$), but the difference between the groups after 48 hours was found to be statistically insignificant ($p > 0.05$). It was determined that different application times did not cause a statistically significant difference in CAT activity ($p > 0.05$) (Fig 2).

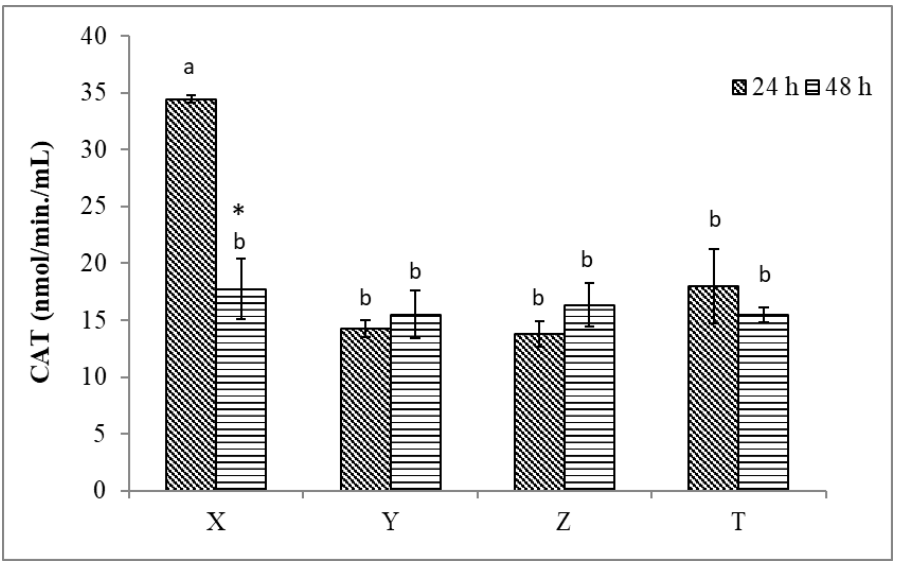

*Figure 2. Changes in CAT activity in P. chrysosporium exposed to rare earth element Ce. The different letters (a, b, c) show the statistical differences between different groups at the same time, and * show the statistical differences between different hours (24th and 48th hours) in the same group.*

3.3. GST activity

GST activities increased statistically in the Y and Z groups at 24 hours, but decreased significantly in the Z and T groups after 48 hours of application compared to the control group ($p < 0.05$)

When the exposure times are compared; statistically significant differences were found between the Z and T groups ($p<0.05$) (Fig 3).

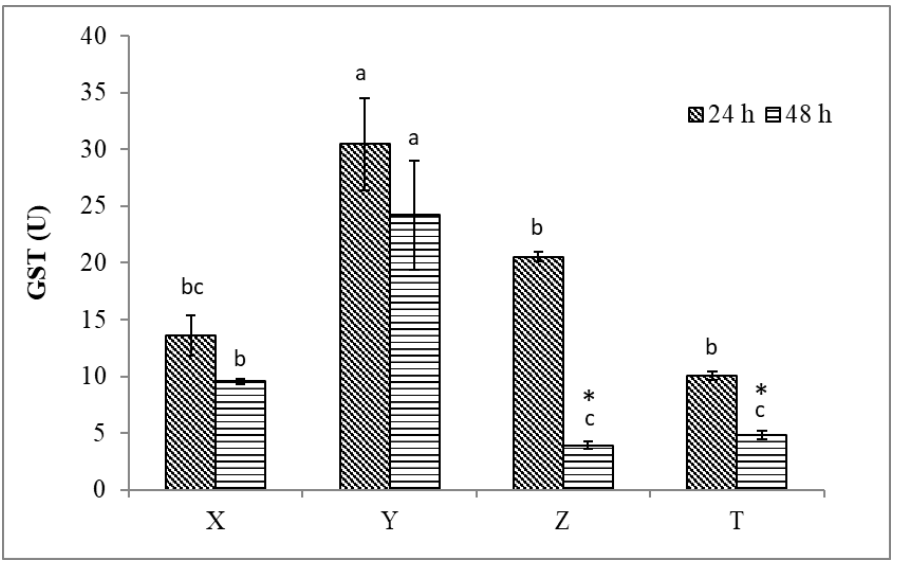

*Figure 3. Changes in GST activity in P. chrysosporium exposed to rare earth element Ce. The different letters (a, b, c) show the statistical differences between different groups at the same time, and * show the statistical differences between different hours (24th and 48th hours) in the same group.*

3.4. GSH levels

GSH levels decreased in all treatment groups after 24 and 48 hours compared to the control group ($p<0.05$).When the exposure times are compared; statistically significant differences were found between the Z and T groups ($p< 0.05$) (Fig 4).

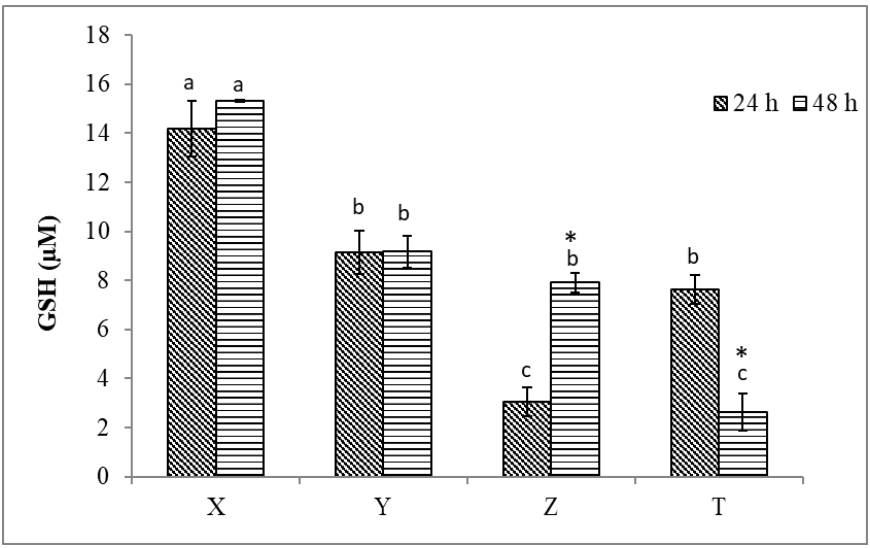

*Figure 4. Changes in GSH levels in P. chrysosporium exposed to rare earth element Ce. The different letters (a, b, c) show the statistical differences between different groups at the same time, and * show the statistical differences between different hours (24th and 48th hours) in the same group.*

3.5. TBARS levels

TBARS levels were found to be increased in Z and T groups compared to the control group after 24 hours ($p < 0.05$). TBARS levels were found to be increased after 48 hours in all treatment groups compared to the control group ($p < 0.05$). No significant differences were found in TBARS levels at different exposure times ($p > 0.05$) (Fig 5).

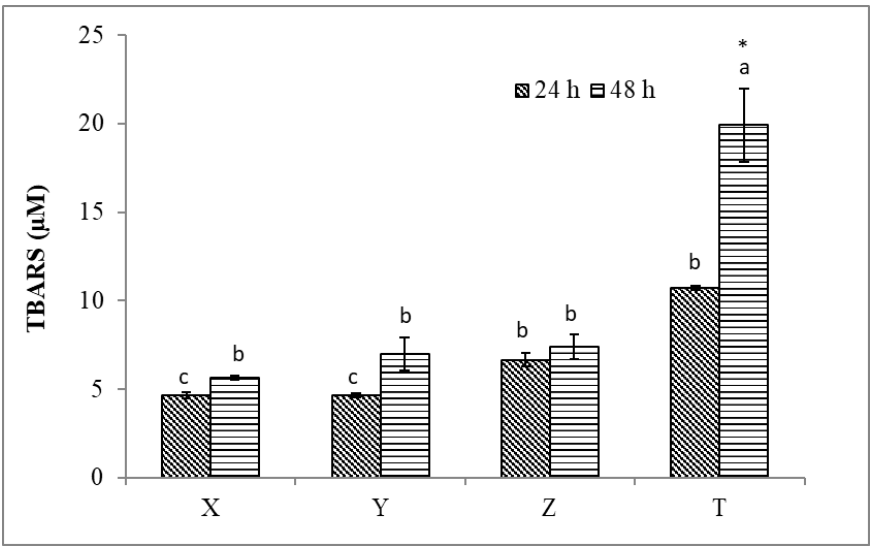

*Figure 5. Changes in TBARS levels in P. chrysosporium exposed to rare earth element Ce. The different letters (a, b, c) show the statistical differences between different groups at the same time, and * show the statistical differences between different hours (24th and 48th hours) in the same group.*

4. Discussion

Due to the numerous applications of Ce ranging from industry to the household, it is necessary to investigate the toxic effects of it. A number of rare earth elements play a role in the electron-exchange reactions in living organisms, causing the generation of free radicals that affect biological processes, e.g., oxidative stress (Todorov et al., 2019). Nowadays, the antioxidant properties of CeO_2 nanoparticles have been discovered (Nelson et al., 2016) It protect cells from damage due to radiation, oxidative stress, or inflammation. Since CeO_2 nanoparticles are capable of stimulating the catalytic potency of SOD, it could be applied as a potent antioxidant (Pagano et al., 2016). Redox properties of CeO_2 nanoparticles could also detoxify

the existing free radicals for prolonged time intervals by maintaining its bioactivity within the tissues (Pourkhalili et al., 2011).

Lipid peroxidation, formation of carbonyls of proteins, enzymes of the antioxidant defense system that act as defense mechanisms in tissues are response parameters that can be used to evaluate the oxidative stress state (Loro et al., 2012). Antioxidant defense system involve enzymes such as SOD, CAT, GST and nonenzymic antioxidant GSH (Aravind and Prasad 2005). The aim of the present study was to make the toxicological assessment of the effect of Ce in *P. chrysosporium* after an acute exposure to this compound.

An increase in reactive species of oxygen and inhibition of several antioxidants following Ce treatments was observed in rice (Xu and Chen, 2011). Increased ROS production and apoptosis induced by Ce were also reported in Taxus tricuspidata plants (Yang et al., 2009). It has been found that the activities of SOD and CAT were induced by rare earth element (Fashui et al., 2002; Nardi et al., 2004). Ippolito et al. (2009) investigated the effect of treatments with La nitrate and a mixture of different rare earth elements on antioxidant systems in common duckweed (*Lemna minor* L.), a floating macrophyte frequently used as model species in biomonitoring and in ecotoxicological studies. They indicated that a remarkable increase in some antioxidant systems was evident in duckweed plants before the appearance of lanthanide-induced toxicity symptoms. Garaud et al. (2015) investigated the lethal effects of CeO_2 nanoparticle on two freshwater invertebrates, *Dreissena polymorpha* and *Gammarus roesel*, using multiple biomarkers. Changes in GPx, CAT and GST enzyme activity were

investigated in *D. polymorpha* and *G. roeseli*. CAT activities in *D. polymorpha* were significantly reduced with $nCeO_2$ exposure at both concentrations compared to control. In gammarids, GST and GPx activities were not significantly affected by $nCeO_2$ exposure. Kumari et al. (2014) investigated the effects of CeO_2 nanoparticles on genotoxicity in female Wistar rats after acute oral exposure. Findings from biochemical analyzes showed that only high-dose CeO_2 nanoparticles had significant changes in ALP and LDH activity in serum and GSH content in the liver, kidneys and brain. Zicari et al. (2018) grew *L. minor* L. plant under laboratory conditions and applied increasing concentrations of Ce ions to the plants in their study. The effects of Ce on plant growth and antioxidant systems were investigated. Increased levels of hydrogen peroxide, antioxidant metabolites, and antioxidant activity confirmed that higher Ce concentrations were toxic to *L. minor.* Correia et al. (2019) used three different ecologically relevant concentrations of CeO_2-NPs (0.1, 0.01 and 0.001 μg/L) for 28 days to characterize chronic toxicity in their study, using biomarkers of Oxidative stress and its effect on neurotoxicity in rainbow trout (*Oncorhynchus mykiss*). GST and CAT enzyme activity were investigated. According to the results, GST enzyme activity increased in the gills of fish exposed to the highest CeO_2-NPs level. In addition, a significant increase in CAT activity was observed in fish livers. Correia et al. (2020) investigated the effects of *Oncorhynchus mykiss* in different organs/tissues (gills, liver and kidney) after acute exposure (96 hours) to 0.25, 2.5 and 25 mg/L CeO_2-NP at three different concentrations. Oxidative stress response (CAT; GSTs, TBARS) were evaluated. Fish exposed to the highest levels had increased

CAT activity in their gills and decreased in their livers. In addition, GSTs and TBARS levels were significantly altered in the analyzed organs. High concentrations of Ce can significantly decrease SOD, CAT, and glutathione peroxidase (GPx) activities and GSH levels, indicating their protective role in balancing damage resulting from oxidative stress (Huang et al., 2011). In the study conducted by Park et al. (2008), oxidative stress caused by cerium oxide nanoparticles in cultured BEAS-2B cells was investigated. Exposure of cultured cells to nanoparticles (5, 10, 20, 40 g/ml) causes cell death, increased ROS, decreased GSH, and induction of oxidative stress-related genes such as catalase, and glutathione. Forest et al. (2017) investigated the effects of CeO_2 nanoparticles on cellular toxicity in vitro. Ce toxicity was evaluated with biochemical parameters such as lactate dehydrogenase release, Tumor Necrosis Factor alpha and ROS production. According to the results, it was found that there was no ROS production, but LDH release and TNF-a production increased significantly depending on the dose. In present study, SOD, CAT activity and GSH levels was decreased compared to the control group. GST activities increased in the Y and Z groups at 24 hours, compared to the control, but decreased significantly in the Z and T groups after 48 hours of application. TBARS levels were found to be increased after 24 and 48 hours. The data obtained from present study show that Ce affects the ROS/antioxidant balance and this is in accordance with already reported data concerning the occurrence of oxidative stress caused by Ce (Liu et al., 2012; Wang et al., 2012a; Wang et al., 2007).

P. chrysosporium has been used as a model organism in toxicity assessment in many study. Demirci and Hamamci (2013)

investigated the antioxidant responses in *P. chrysosporium* exposed to Astrazone Red FBL textile dye. They showed that the ability of *P. chrysosporium* to antioxidative response and defence system exposed to Astrazone Red FBL. Yildirim et al. (2019) used *P. chrysosporium* as a model organism to assess the toxicity of municipal landfill leachate from Elazığ, Turkey. They found that SOD activities were decreased in the application groups compared with the Control Group at the 24th and 96th hours. CAT activities and GSH and MDA levels increased. Wan et al. (2015) investigated the lead-induced oxidative stress toxicity of *P. chrysosporium* in their study. The results showed that *P. chrysosporium* can adapt to lead-induced stressful situations by regulating the oxidant-antioxidant process and maintain the balance between oxidants and antioxidants. Zeng et al. (2012) investigated the response of *P. chrysosporium* to toxic pollutants in their studies. It was observed that the formation of reactive oxygen species and antioxidant levels increased after cadmium application. SOD activity at low cadmium concentrations correlated well with malondialdehyde levels. However, this correlation was found to decrease and malondialdehyde levels increased significantly at the highest cadmium concentration tested. In the study conducted by Chen et al. (2014) plasma membrane behavior, oxidative damage and defense mechanism were investigated in *P. chrysosporium* under Cadmium stress. The results showed that cadmium causes solidification of lipids, a decrease in H^+-ATPase activity, and plasma membrane damage, including lipid peroxidation. It has been suggested that cellular death may be mediated by mitochondrial membrane destruction and generation of reactive oxygen species, as well as by

oxidative stress. In present study, it was demonstrated that *P. chrysosporium* can be used as a model organism in the evaluation of the toxic effect of Ce.

5. Conclusion

The results obtained from the study showed that Ce has a toxic effect on *P. chrysosporium.* This fungus can be used as a model organism in the evaluation of the toxic effect of cerium. SOD, CAT, GST activities and GSH, TBARS levels have been shown to be suitable biomarkers in the evaluation of the toxic effects of Ce. It has also been observed that the application times and application concentrations have a toxic effect in different levels on the model organism *P. chrysosporium.*

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CHAPTER IV

Evaluation of the Toxicity of Zinc and Zinc Oxide Nanoparticles in Terms of Metallothionein in *Daphnia Magna*

Yeliz ÇAKIR SAHİLLİ

Introduction

Nanoscale materials, defined as single-phase or multiphase particles (NPs) with crystal sizes ranging from 1-100 nm in at least one dimension, have long been used in science and technology. Their use has been going on since ancient times (Delatte, 2001; Sanchez & et al., 2003). NP metals, which are an important source of oxidative stress formation, play an important role in biological systems. Due to their small size and large specific surface area, metal oxide NPs have a high willingness to undergo chemical reactions. This leads to damage in cells and organelles, causing hepatotoxic toxicity through direct or metabolic pathways. For all these reasons,

most nanotoxicity studies have focused on nanoscale metal oxides (Wang & Fowler, 2008).

Because zinc is widely used in industry, it is commonly found in nature, especially in areas where industry is developed. Zinc waste enters surface waters in these area (Bunker & et al., 1984; Thomas & et al., 1988).

Nanoparticles (NPs), which are constantly evolving due to their physical and chemical properties, have very important potentials in aquatic and terrestrial (Vance & et al., 2015; Martin, Telgmann & Metcalfe, 2017). Studies on the toxicity of NPs in aquatic organisms are rapidly increasing and environmental risk assessments of these substances are also considered as important studies (Garner & et al., 2015; Martin, Telgmann & Metcalfe, 2017). The production of metal or metal oxide NPs is increasing rapidly in the world and as a result, their access and contamination of aquatic and terrestrial ecosystems are increasing (Gottschalk & et al., 2009; Morales-Diaz & et al., 2017). NPOs can cause adverse effects on the lives of other living organisms. Therefore, the removal of such pollutant ions is extremely important for the protection of human life and the environment. However, the interaction of NPs with the environment they reach after contamination and exposure times is important for their evaluation (Ben-Moshe & et al., 2013; Aruoja & et al., 2015).

ZnO NPs are widely used as additives. Antibacterial effect due to the production of sunscreens, cosmetics, textiles, pigments, catalysts and catalytic converters. They are used in many fields such as paints (Nowack & Bucheli, 2007; Becheri & et al., 2008). More

importantly, ZnO NPs have been proposed as suitable anticancer agents for the treatment of certain types of cancer and water disinfection as they exhibit selective toxicity (Hanley & et al., 2008; Lin & Xing, 2008).

State of the art in ecological toxicological testing and to obtain meaningful results, not only the appropriate test type but also the appropriate test organism should be selected (Rand, 1995). The most important factor in bioassay or toxicity studies is the selection of organisms. In this study, the freshwater flea *D. magna* was selected. *D magna* is a good sentinel species for aquatic organisms. This species is sensitive, easy to culture and has a short generation time of about 8-10 days in the laboratory.

MT was first identified in 1957 by Margoshes and Valle as a Cd-binding protein at isolated from the kidney (Margoshes & Vallee, 1957). MTs are low molecular weight, cysteine-rich, cell is an antioxidant protein known to be involved in proliferation, apoptosis, homeostasis of essential metals and metal detoxification. The biological functions of MTs, which show high affinity for metals, have not been fully elucidated despite their discovery many years ago. MTs are present in different groups of living organisms such as prokaryotes, bacteria, invertebrates, vertebrates and aquatic organisms (Roesijadi, 1992; Newman, 2009). MTs, which are usually found in the cytosol, are a low molecular weight (6-7 kDa), metal-binding, cysteine-rich protein, lacking aromatic amino acid compounds and composed of metal thiolate groups [Szczurek, and Bjornsson, 2001; Newman, 2009). MTs exist in a number of isoforms of the protein with different amino acid composition and charge, depending on the species. Metallothionein has been found in

many tissues of organisms, including the brain, thymus, bone marrow and reproductive organs (Bremner & Beattie, 1990). MTs are composed of 20 cysteine residues such as lysine, serine and arginine and are thought to have a role in metal binding (Mitropoulos & et al, 2005). The MT mechanism is thought to regulate the immune system, detoxify, catalyze and store harmful substances (Webb, 1987). Although MTs have binding power among 18 different metal molecules, they can bind Cu, Cd, Zn, Pb, Ag more (Coyle & et al., 2002).

In this study, MT level changes in *D magna* exposed to metal-based Zn and ZnO NPs were measured semi-quantitatively. The aim of the study was to determine the changes in MT biomarkers of Zn and ZnO NPs at 24 and 96 hours and to evaluate the acute effect of MT activity.

Material Method

Nanoparticles

The NP materials Zn (40-60 nm) and ZnO (10-30 nm) used in the study were obtained from commercial companies. Chemicals from the analytical reagent class were used without any purification or purification. For both NPs, the shape and size data declared by the manufacturer were taken as reference and used in the bioassay studies.

Bioassay Organism

Daphnia samples used in our study were obtained from local aquarists. Spontaneous deaths were minimized by applying a 15-day adaptation period to the test animals brought to the laboratory environment. In the vital activities of living thingsroutinely fed to

avoid any disruption and also photoperiod (16:8 hours light/dark) was applied. Acute toxicity testing was performed according to OECD 202 instructions.

Preparation of Nanoparticle Suspensions

10% (m/v) stock suspensions of Zn and ZnO NPs in ultrapure water with (18.0 MΩ) in a vortex for 5 minutes. Each stock solution was homogenized by vortexing for 5 minutes and then homogenized for an average of 15-20 minutes to ensure the highest NP dispersion sonification was carried out in an ultrasonic bath. The stock of each NP suspension, taking into account the concentration ratios specified in the experimental design, appropriate volumes were taken with the help of an automatic pipette and without wasting time groups of organisms were exposed to NP.

Bioassay Setup

Control in which NPs were not applied and the optimum living environment of the experimental organism was provided group was created. NP application concentrations, considering the release rates to the environment and determined at low rates. Application on the effect of NPs on *D. magna* concentrations are indicated in Table 1. Each aquarium at the beginning of the experiment The experimental design was designed by placing 15 individuals in it. Control and treatment groups were carried out independently of each other with 3 replicates.

Table 1. Bioassay design of organisms exposed to Zn and ZnO nanoparticles

Grups	Control	A	B	C
NP-Zn/ZnO (ppm)	0	10 mg/L	20 mg/L	40 mg/L
D. Magna	15	15	15	15

Metallothionein Analysis

In the study, Biosense brand ELISA kit was used. This kit contains the MT biomarker a series of Enzyme Linked Immunosorbent Assays to be used for semi-quantitative detection reagent. The method is based on biomarker detection using a suitable monoclonal or polyclonal antibody in an indirect antibody capture ELISA format.

Statistical Analysis

All experiments in the study were repeated independently in triplicate and the data were recorded as means with standard deviation. Statistically significant difference between the groups significant differences one-way analysis ANOVA, SPSS/24.0 package program TUKEY multiple comparisons.

Results

The effects on D. magna exposed to Zn and ZnO, with MT levels expressed as a percentage of control values, are presented in Figures 1 and 2.

Statistically significant differences ($p< 0,05$) were detected in *D. magna* exposed to Zn-NP at 24 and 96 hours compared to the control (Figure 1). For Zn-NP, there was a linear increase in MT

levels depending on the exposure time and increasing concentrations.

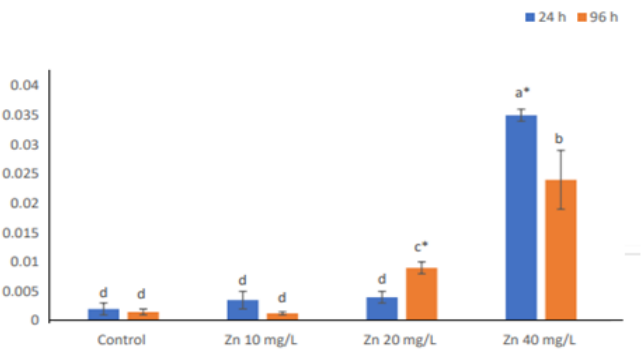

Figure 1. MT levels in D. magna exposed to Zn-NP: Different letters on the columns indicate statistical differences between groups, Asterisk (*) sign indicates statistical differences between different hours of the same group.

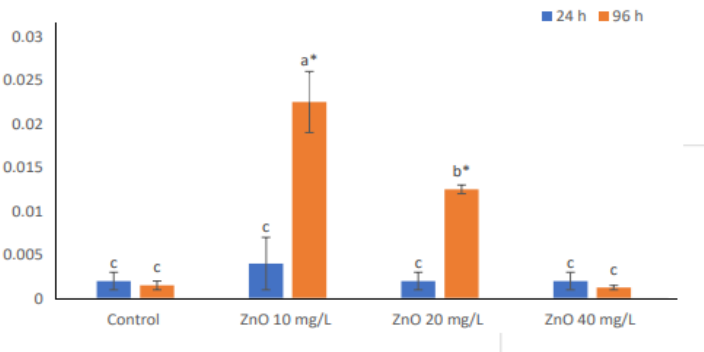

Figure 2. MT levels in D. magna exposed to ZnO-NP: Different letters on the columns indicate statistical differences between groups, Asterisk (*) sign shows the statistical differences between different hours of the same group.

In *D Magna* exposed to ZnO-NP, there was a statistically significant increase ($p<0.05$) in MT levels in the 10 and 20 mg/L treatment groups for 96 hours and no statistically significant difference ($p>0.05$) in the 40 mg/L treatment group compared to the control (Figure 2). Considering the exposure time of the groups to ZnO-NP, a statistically significant increase ($p<0.05$) was found between the 10 and 20 mg/L application groups (24-96 hours), while no statistically significant difference was found in the MT levels of the other application groups ($p>0.05$).

Discussion

In this study, it was aimed to investigate the effects of Zn-NPs and ZnO-NPs on the aquatic ecosystem in case of their possible introduction into the aquatic ecosystem, their responses to MT biomarkers in a model organism *D magna* and their potential use in ecotoxicological studies. Non-lethal concentrations of both NPs were determined in preliminary studies.

Although the difference between MT-metal components between living organisms and tissues varies according to the type of metal exposed and the mode of exposure, the interest of MTs in binding different metals can be ranked as $Zn^{+2}<Pb^{+2}<Cd^{+2}<Cu^{+2}<Ag^{+2}= Hg^{+2}=Bi^{+3}$ (Thomas, Bachowski & Girotti, 1986; Vašák, 2005). Tissues directly involved in metal uptake, storage and excretion have a high MT synthesis capacity (Amiard & et al., 2006). Although some of the essential metals are essential for living organisms, high concentrations of can be toxic when they reach (Torres, Tort & Flos, 1987).

Hogstrand et al. investigated the role of metallothionein protein in metal binding by exposing freshwater perch (Perca fluviatilis) to Cu, Zn and Cd (Hogstrand, Lithner & Haux, 1989). According to the findings of the study, they reported that there was a positive correlation between Zn levels and MT levels. Van Campenhout et al. investigated Cd and Zn metal accumulation and MT levels in rockfish. They reported that there was a positive correlation between Cd and Zn and MT levels (Van Campenhout, Bervoets & Blust, 2003). Bervoets et al., they investigated Cd, Cu and Zn levels and MT induction in stream rockfish (Gobio gobio), rudd (Rutilus rutilus) and perch (Perca fluviatilis) (Bervoets & et al., 2013). They reported a positive correlation between MT levels and hepatic zinc levels. In a study, Cu, Cd, Zn and MT levels of mullet (*Leuciscus cephalus*) and sea bass (*Perca fluviatilis*) caught from nature were determined. Since the heavy metal levels of the fish they caught were below the legal limits, they suggested that there was no change in MT values related to heavy metals. In general, when high concentrations of non-essential or essential metals are present in aquatic organisms, MT concentration in cells increases, reflecting the high metal bioavailability in the environment (Shariati & Shariati, 2011; Geffard, Amiard & Amiard-Triquet, 2002a ; Geffard, Amiard-Triquet & Amiard, 2005; Amiard & et al., 2006).

Zhang and Wang reported a significant increase in MT levels in *Acanthopagrus schlegeli* exposed to Zn (Zhang & Wang, 2005). Mosleh Yahia et al. exposed *Tubifex tubifex*, an aquatic invertebrate organism to different copper concentrations for 7 and 15 days. MT levels in exposed organisms were significantly increased in relation to the duration of exposure at different copper concentrations

(Mosleh, Parıs-Palacıos & Bıagıantı-Rısbourg, 2006). Khati et al. reported a correlation between the induction of MTs in *Perna perna mussels* exposed to Cd and Cu (200 µg/l, 30 µg/l). Similarly, metallothionein induction in various aquatic organisms contaminated with metals has been reported in scientific studies (Zhang & Schlenk, 1995; Pourang & Dennis, 2005; Khati & et al., 2012).

Increased MT contents are considered a specific biomarker of metal exposure. Mao and et al. the general structure and function of MT, gene structure, transcription regulation, induction factors and reported that heavy metals affect the development of aquatic invertebrates (Mao, Wang & Yang, 2012). In a study on the toxic effect of Zn and ZnO nanoparticles on Artemia salina and Daphnia magna, it was stated that the biochemical and toxicological effects of NPs are not fully understood because the observed effects depend on the size of the nanoscale material, physico-chemical properties and organismal diversity. In this study, Zn (40-60 nm) and ZnO (10-30 nm) NPs were used and it was found that MT levels reached maximum at 10 and 20 mg/L concentrations of ZnO NPs. For ZnO NP bonding MT concentration increases and after reaching a certain capacity at high concentration, MT synthesis capacity is estimated to decrease. In this study, similar to the studies in the literature Increasing concentrations of Zn-NP and ZnO-NP increased compared to the control.

Useful results can be obtained by using *D magna* as a biological matrix for MT levels in monitoring programs for MT biomarkers of exposure to metallic NPs Zn-NP and ZnO-NPs. A control mechanism should be established for the use and release of

these NPs into the environment, and comprehensive studies should be conducted on their effects in different organisms, such as cytotoxicity, genotoxicity and histopathology.

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CHAPTER V

Oral Biofilms and Nanocontrol

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Introduction

Microorganisms naturally exist in highly complex, heterogeneous structures that are called biofilms. Biofilm as being a multiple microbial species community of sessile microbes can be formed on various surfaces and their properties are fundamentally different from those of microbes in planktonic suspensions (Ray et al., 2024).

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Biofilm formation and development is a dynamic process in itself (Hall-Stoodley et al., 2002; Nadell et al., 2008; Kostakioti et al., 2013). When a planktonic microorganism attaches to a surface, it can join with other microorganisms to form a biofilm structure. In this respect, each microorganism has its own specific method for adhesion. Examples of these include flagella, pili, proteins and polysaccharide adhesins (Berger et al., 2018).

Since microorganisms aggregate on both biotic and abiotic surfaces and form biofilms, there is a difficulty in identifying and isolating them as a therapeutic target (Morse et al., 2018). One of the most critical stages for biofilm formation is the initial attachment of the bacterial cell. After the bacteria adhere to the surface, depending on the environmental conditions, the bacteria have two options; first, they can proceed to biofilm formation by adhering to the surface or, as an alternative, they can return to their planktonic phase (Berger et al., 2018)

Biofilms cause many bacterial infections, and the long-term presence of biofilms causes an inflammatory response that is difficult to repair by the immune system, so resulting in difficult chronic infection processes (Yang et al., 2011; Burmølle et al., 2014; Vestby et al., 2020). The mechanisms and life cycle of biofilm formation can be broadly described in five main steps: initial attachment, irreversible attachment, microcolony formation, biofilm maturation and dispersion (Figure 1) (Liu et al., 2024).

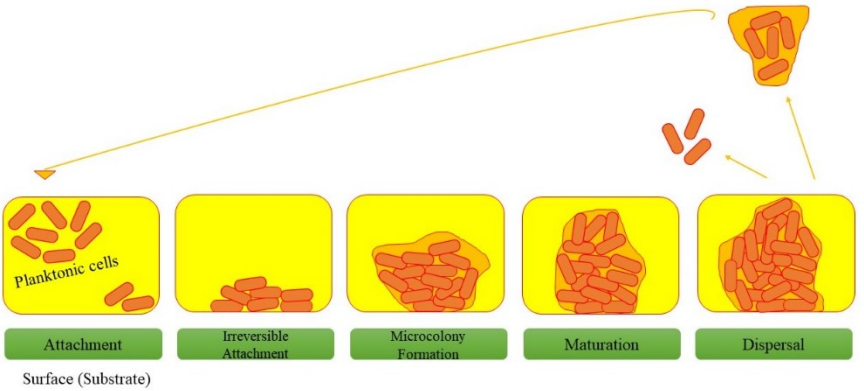

Figure 1: The lifecycle of a biofilm structure (Liu et al., 2024).

Biofilms can be formed as monomicrobial or polymicrobial. While monomicrobial biofilm layers refer to the microbial community consisting of a single microorganism species, polymicrobial biofilms refer to the biofilm structure formed by many microorganism species with different properties (Ferraz, 2024). In comparison, polymicrobial biofilms have been reported to tend to be more resistant than monomicrobial biofilms. In addition, polymicrobial biofilms have different architectural structures due to symbiotic interactions between different types of microorganisms in the biofilm structure. Such biofilms have varying tolerance to antimicrobial agents and restricted environmental conditions (e.g., limited nutrient source, host immune system) (Price et al., 2020). Polymicrobial biofilms are among the main factors that cause oral, wound, respiratory, skin and bone infections (Anju et al., 2022).

Biofilms consist of many microorganisms that form a multicellular structure. The biofilm complex is held together by a matrix called extracellular polymeric compounds (EPS). This matrix structure, mostly water, provides protection, adhesion, stabilization,

and nutrients to the biofilm complex (Jass et al., 2003). The microbial content of the biofilm is approximately 5%, the EPS matrix accounts for 2%, and DNA, RNA, and proteins account for another 2% (Table 1). The components of the matrix structure vary depending on the bacterial elements and environmental conditions (Berger et al., 2018; Rather et al., 2021).

Table 1: Biofilm composition (Rather et al., 2021)

Components	Percentage (%)
Microbial cells	2–5
Water	Up to 97
Polysaccharides	1–2
Proteins (including enzymes)	< 1–2
Nucleic acid (DNA and RNA)	< 1–2

Biofilm and Antibiotic Resistance

Dental biofilms represent a worldwide health concern which affect the 80% of the population. The plaque formation on the tooth surface gets adherent over time if it is not treated in an appropriate method (Dash and Ragavendran, 2024).

The biofilm structure is more resistant to therapeutic treatments and immune system responses compared to planktonic bacteria because it is protected by a matrix of organic polymers produced by the biofilm (Costerton et al., 1987).

Elements that constitute the multifactorial defense system of the biofilm include the formation of resident cells, the development of adaptive stress responses, limited and less antibiotic penetration, limited nutrition, reduced growth and metabolic activity (Stewart, 2002), and the inactivation of antimicrobials within the components of the EPS matrix (Hall and Math, 2017; Rather et al., 2021).

The effectiveness of mechanical or chemical treatments applied for hygiene purposes is quite limited due to the difficulty of accessing the interdental spaces (Schmidt et al., 2013). Therefore, alternative treatments with strong antibiofilm activity need to be investigated (Villa et al., 2020).

Oral Microflora and Biofilms

Oral cavity provides an excellent opportunity for biofilm formation due to its rich nutrients and the presence of favorable environments for microorganisms (Figure 2). Biofilm formed on the tooth surface leads to caries, which eventually leads to diseases such as periodontitis and eventually tooth loss (Ray et al., 2024). As a result of intensive researches, it has been reported that various systemic and chronic diseases are associated with the imbalance of the oral microbiota and the intervention of periodontal pathogens (Ray, 2023); therefore, appropriate control and management of the biofilm layer formed in the oral environment is important in terms of many factors.

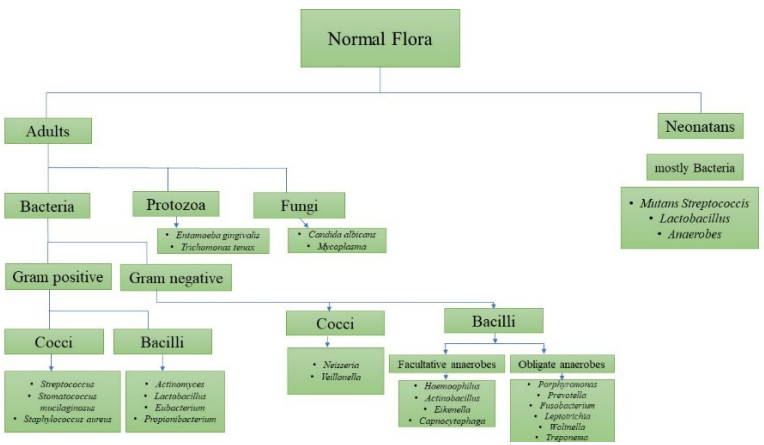

Figure 2: Normal oral flora (Patil et al., 2013).

Dental plaque is a polymicrobial biofilm layer found in the mouth that can cause infections such as caries and periodontal disease (Morse et al., 2018; Jakubovics et al., 2021) . The oral cavity is a unique habitat in terms of microbial diversity and can host up to 1000 different species of microorganisms (Morse et al., 2018). Biomedical supports, including dentures and implants, are also suitable substrates for biofilms, as are natural teeth (Berger et al., 2018).

Dental caries is a biofilm-induced healthcare proble resulting in the damage of mineralized tooth tissue (Takahashi et al., 2011; Koo et al., 2013; Pitts et al., 2017). Oral microorganisms are a causative agent of dental caries but are not sufficient alone, because the formation of cariogenic biofilms is directly related to the host's diet (Takahashi et al., 2011; Pitts et al., 2017). There are diverse types of microorganisms in dental biofilm layer that adhere on to the surface of teeth or dental biomaterials like implants (Flemming et al., 2016). *S. mutans*, a member of the mutans streptococci (MS) group, is one of the most prominent gram-positive bacteria associated with dental caries (Hajishengallis, et al., 2017). Sucrose is essential for the success of the organism as a pathogen, as *S. mutans* converts sucrose into extracellular insoluble glucans that enhance bacterial adhesion-cohesion and also EPS matrix (Bowen et al., 2011). *S. mutans* interacts with other microorganisms in a dynamic and coordinated polymicrobial complex to form a cariogenic biofilm (Simón-Soro and Mira, 2015; Bowen et al., 2018)

The most prevalent oral diseases (Figure 3) such as caries, periodontitis, and peri-implantitis are directly related to the dental biofilm, which is mainly formed by *Actinomyces*,

Lactobacillus, *Porphyromonas*, *Streptococcus* and fungal strains (Dhanasekaran et al., 2014; Kouidhi et al., 2015; Matsumoto-Nakano, 2018).

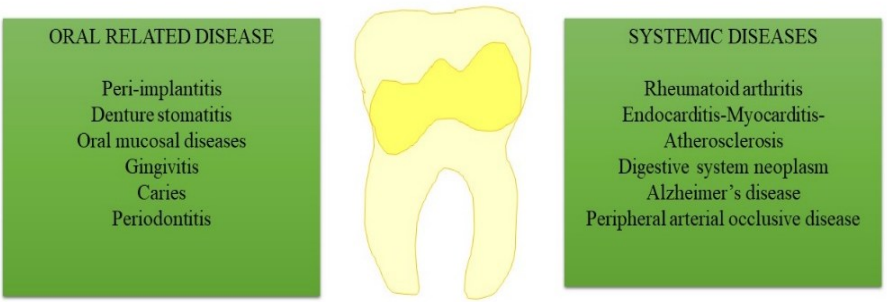

Figure 3: Dental biofilm and its associated diseases (Kannan et al., 2024).

Denture wearers show increased *Candida albicans* biofilm density because *C. albicans* is a pathogenic fungus that colonizes the denture material polymethyl acrylate (PMA). Such prevalence of *Candida* in the mouth increases the risk of developing denture stomatitis, a type of inflammation of the oral tissue, in denture wearers (Susewind et al., 2015). Approximately 65% of denture wearers develop stomatitis, which contributes to poor oral health, poor dental hygiene, and systemic diseases including diabetes (Preshaw et al., 2011).

Control of Oral Biofilm

Although biofilms are difficult to completely eliminate, there are several traditional strategies for control. Methods for removing oral biofilms include mechanical or physical methods such as tooth brushing. However, researchers has figured out that mechanical

brushing alone is not sufficient to remove biofilms and that a cleaning agent must be added to the process (Berger et al., 2018).

Studies have shown that mouthwashes with antiplaque function are effective against biofilms [17]. Although these mouthwashes are effective in removing plaque, mouthwashes containing chlorhexidine gluconate, as well as mouthwashes containing essential oils, have undesirable side effects. Tooth discoloration and occasional loss of taste are side effects that may develop due to the use of chlorhexidine gluconate. Essential or volatile oils seem to be preferred because they do not have these undesirable side effects and have low mammalian toxicity (Berger et al., 2018). Stoeken et al. (2003) published a review on the effects of a mouthrinse containing essential oils (EO) on plaque and parameters of gingival inflammation ans resulted that EO has an additional benefit with regard to plaque and gingivitis reduction.

Nanoantimicrobials with antibiofilm activity

Conventional methods such as mechanical removal and chemical agents used for biofilm control are often unsuccessful in completely eliminating biofilms, thus leading to recurrent oral infections and diseases. Nanotechnology has recently become a frequently used solution as a promising approach to overcome such challenges by providing new strategies for drug delivery and biofilm disruption (Kaushal et al., 2024; Dash et al., 2024).

Nowadays, drugs produced and formulated through nanotechnology are of great interest in the treatment of dental biofilm due to their nano-sized dimensions that penetrate and disrupt

the cell membrane and extracellular polymeric substance (EPS) matrix of pathogens (Zainab et al., 2022; Reddy et al., 2023).

Nanoparticles are increasingly important therapeutic approaches due to their capacity to deliver active ingredients to the target area in appropriate dosage, protection against deactivation and providing more activity with fewer side effects. (Qayyum & Khan, 2016; Liu et al., 2019; Wang et al., 2020; Al-Wrafy et al., 2022). Formulations containing nanoparticles have high selectivity against microorganisms and have the capacity to overcome biological barriers, such as biofilm structure, due to their small size, large surface area and highly reactive structure. (Blanco et al., 2015; Sharma et al., 2019; Al-Wrafy et al., 2022). The dimensions of NPs, as the name suggests, are small enough to overcome the microbial cell wall and biofilm structure. Their large surface area makes it easier to load drugs into nanoparticles (Ramasamy and Lee, 2016).

Metals have been used as antimicrobial agents since ancient times. Silver, copper, gold, titanium and zinc have received particular attention because they each have different characteristics and activity (Allaker and Yuan, 2019). Studies show that there is an inverse relationship between the size of nanoparticles and antimicrobial activity, such that particles with a size range of 1–10 nm exhibited the greatest biocidal activity against bacteria (Morones et al., 2005; Verran et al., 2007). Some nanoparticles, due to their very small size, may have an advantageous use in the biomedical field which may lead to improved biocompatibility (Kim et al., 2007). In particular, inorganic nanomedicines have attracted considerable attention with their antibiofilm activity (Chong et al.,

2022). Among them, silver nanoparticles have the potential for medical use as alternative antimicrobial agents (Meher et al., 2024). Studies have reported that silver nanoparticles tend to inhibit bacterial growth when used in prostheses, materials and implants (Rai et al., 2014; Rai et al., 2014).

Advances in nanomedicine technology are promising in preventing the development of oral biofilms. Nanoparticles such as silver, chitosan, gold, and titanium have shown to be effective in combating biofilms. However, due to the need for standard protocols, safety concerns, and regulatory approval challenges, the clinical use of nanoparticles has not gained momentum. New researches should focus on further exploring the potential of nanotechnology in combating oral biofilms and improving oral health.

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CHAPTER VI

Microalgal Biofertilizers: A Sustainable Solution for Modern Agriculture

Deniz ŞAHİN[1]

1. Introduction

In recent years, the need for an increase in agricultural production, especially in parallel with population growth, has necessitated the intensive use of fertilizers. However, excessive and uncontrolled use of chemical fertilizers has led to serious environmental problems such as soil and water pollution, loss of biodiversity and contribution to climate change. Chemical fertilizers cause damage to the environment by accumulating in soil and water resources, while only 50% of the applied amount is taken up by plants (Renuka *et al.*, 2018; Cao *et al.*, 2023).

In this context, microalgal biofertilizers attract attention with their potential to both increase agricultural productivity and support environmental sustainability. Microalgae reduce the carbon footprint by converting atmospheric carbon dioxide (CO_2) into organic compounds thanks to their photosynthetic capacity, while at the same time increasing soil fertility through mechanisms such as nitrogen fixation and phosphorus solubilisation (Zhu *et al.,* 2021).

The success of microalgae in agricultural applications is not limited to their environmental advantages. These organisms make essential nutrients such as nitrogen, phosphorus and potassium available to soil and promote plant growth by producing bioactive substances such as phytohormones, polysaccharides and antimicrobial compounds (Wuang *et al.,* 2016; Chittora *et al.,* 2020).

Furthermore, unlike chemical fertilizers, microalgal biofertilizers enrich the soil microbiota, creating a healthier ecosystem. This improves long-term soil fertility while also helping to reduce environmental pollution (Zhang *et al.,* 2024).

As a result, microalgal biofertilizers are emerging as an important tool to mitigate the environmental impacts of chemical fertilizers and to provide a sustainable solution in agricultural production. In this study, biological properties, production methods, agricultural applications and environmental impacts of microalgal biofertilizers will be discussed in detail.

2. Properties of Microalgae

2.1. Taxonomy and Diversity

Microalgae are a large group of organisms including many classes such as *Chlorophyceae*, *Cyanophyceae*, *Bacillariophyceae* and *Chrysophyceae*. Species in this group are widely distributed in freshwater, saltwater and terrestrial ecosystems. Species such as *Chlorella*, *Spirulina*, *Scenedesmus* attract attention in agricultural and environmental applications with their ability to grow rapidly and adapt to different environmental conditions (Bello *et al.*, 2021). These microalgae species have the potential for wide use as biofertilizers due to their biodiversity and high biomass production capacity.

2.2. Biochemical Composition

The biochemical composition of microalgae is very rich and plays an important role in agricultural applications. In general, microalgae contain essential components such as protein (50-60%),

carbohydrates (10-30%) and lipids (5-25%), as well as pigments (chlorophyll, carotenoids), phytohormones and polysaccharides (Bello *et al.,* 2021).

Phytohormones, especially auxin and cytokinins, promote plant growth, while polysaccharides improve soil structure. For example, species such as *Chlorella vulgaris* and *Spirulina platensis* are rich in these components and provide bioactive contributions for agricultural production (Tate, 2013).

2.3. Carbon Sequestration

Thanks to its photosynthetic capacity, microalgae can perform carbon sequestration by converting atmospheric CO_2 into organic compounds. This feature of microalgae is recognised as an important tool in combating climate change and reducing carbon footprint. Especially *Chlorella vulgaris* and *Spirulina* species stand out with their high carbon sequestration capacity. Research shows that microalgae can bind about 180 tonnes of CO_2, producing 100 tonnes of biomass per hectare per year (Dagnaisser, 2022).

The biological carbon capture ability of microalgae offers both economic and environmental benefits in sustainable agricultural systems, increasing the future application potential of these organisms.

3. Mechanisms of Action

3.1. Nutrient Cycle

Microalgae support soil nutrient cycling through nitrogen fixation and phosphorus solubilisation, making essential nutrients more accessible to plants. *Cyanobacteria*, in particular, increase the soil nitrogen pool by fixing atmospheric nitrogen and converting this nitrogen to ammonia through biological processes. *Cyanobacteria* with heterocysts such as Anabaena and Nostoc play an important role in this process. By fixing nitrogen, cyanobacteria both improve the nitrogen balance of the soil and increase agricultural productivity in the long term (Alvarez *et al.,* 2021; Song *et al.,* 2022).

In addition, microalgae make soil phosphorus available to plants by secreting organic acids that increase phosphorus solubility. This process is particularly important in soils rich in phosphorus but biologically inaccessible. This function of microalgae improves soil fertility and reduces the use of chemical fertilizers (Coppens *et al.,* 2016).

3.2. Stimulating Plant Growth

Phytohormones produced by microalgae are biochemical compounds that promote plant growth. Hormones such as auxins (e.g. indole-3-acetic acid), cytokinins and gibberellins accelerate plant root and stem growth, promote cell division and increase photosynthesis. For example, extracts from *Chlorella* and *Spirulina* species contain these hormones, favourably affecting both root and stem growth in plants (Lu *et al.,* 2015).

Furthermore, phytohormones increase the resistance of plant tissues to abiotic stress conditions. This makes microalgae particularly useful for plants grown in saline or arid soils. These hormones positively influence plant metabolism through the transport of microalgae extracts to the plant via foliar spraying or soil applications (Song *et al.,* 2022; Tiwari *et al.,* 2019).

3.3. Improving Soil Health

Microalgae support soil health through organic matter production and improvement of microbial community structure. Polysaccharides released as a result of cellular metabolism of microalgae improve soil structure by increasing organic carbon accumulation in the soil. In particular, exopolysaccharides (EPS) increase soil microbial activity and improve soil water holding capacity and air permeability (Renuka *et al.*, 2018; Marks *et al.,* 2019).

In addition, microalgae have positive effects on soil microbiota. By increasing microbial diversity in the soil, it supports plant-microorganism interactions and contributes to the long-term fertility of the soil. This process ensures more efficient utilisation of

nutrients such as nitrogen, phosphorus and potassium in the soil (Ronga et al., 2019; Tiwari et al., 2019).

These mechanisms support the use of microalgae as effective biofertilizers that promote environmental sustainability. In particular, nitrogen fixation, phosphorus solubility, phytohormone production and their contribution to soil health make microalgae a valuable option both economically and environmentally.

4. Production and Formulation

4.1. Breeding Systems

The most widely used systems for microalgae production are open ponds and closed photobioreactors. While open pond systems are favoured for large-scale production due to their low cost and simple structure, they have the disadvantages of being more sensitive to contamination and low biomass yield. In these systems, paddle wheels are usually used for mixing the water. However, environmental factors such as temperature control and light distribution are difficult to regulate (Benemann and Oswald, 1994; Bello *et al.*, 2021).

Closed photobioreactors offer advantages such as higher biomass yields and reduced risk of contamination. These systems enable tight control of environmental factors such as light and temperature. However, high installation costs and technical complexity limit their large-scale application (Tredici, 2009).

Hybrid systems offer an approach that combines the advantages of open and closed systems. In these systems, the initial stages of microalgae are produced under sterile conditions in closed reactors, while the final stages are completed more economically in open ponds (Borowitzka and Vonshak, 2017; Liu *et al.*, 2019). Figure-1 illustrates the different cultivation systems, including open ponds, closed photobioreactors, and hybrid systems, which are used for microalgae production.

4.2. Wastewater Utilisation

Wastewater provides an economically and environmentally sustainable environment for the cultivation of microalgae. Rich in nutrients such as nitrogen and phosphorus, wastewater promotes the growth of microalgae while contributing to the biological treatment of waste. The use of wastewater can reduce microalgae production costs by up to 67%, and this method offers a particularly effective way to utilise municipal and industrial waste (Acién *et al*., 2018; Wang *et al.,* 2010; Costa, 2021).

The utilisation of wastewater in microalgae production not only reduces production costs, but also contributes to the reduction of greenhouse gas emissions. This approach enables the recovery of nutrients such as nitrogen and phosphorus while at the same time preventing pollution (Kiran *et al*., 2014; Malik *et al*., 2022; Razzak *et al.,* 2013)

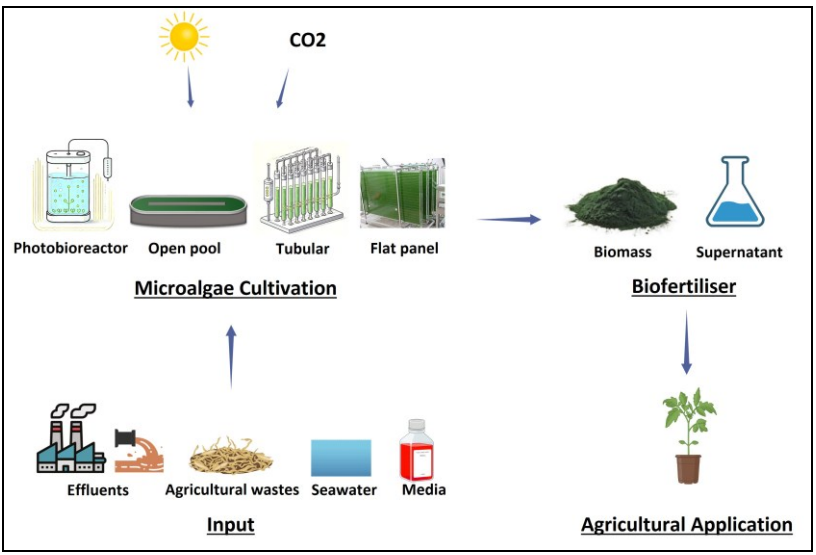

Figure 1: Microalgal cultivation systems for biofertilizer production. Examples include open-pond systems, closed photobioreactors, and hybrid systems used to optimize biomass production and nutrient recovery.

4.3. Formulation Types

Biofertilizers from microalgae can be prepared in different forms such as liquid extracts, granules and coated products. Liquid extracts can be used as foliar fertilizer, while granules increase soil fertility by providing prolonged release (Benemann and Oswald, 1994; Costa 2021).

Coated microalgae products have been developed specifically to ensure slow release of nutrients and to increase resilience to environmental conditions. These products offer an environmentally friendly alternative to chemical fertilizers in organic and conventional farming practices (Razzak *et al*., 2013; Qin *et al*., 2019).

These production and formulation methods enable the large-scale use of microalgal biofertilizers in agriculture and promote environmental sustainability. Especially hybrid systems and the use of wastewater attract attention with their economic and environmental advantages.

5. Agricultural Applications

5.1. Increasing Product Yield and Quality

Microalgal biofertilizers have significant potential to improve yield and quality in many plant species. For example, studies in crops such as tomato, lettuce and wheat have shown that microalgae applications increase fruit yields, support plant root development and improve soil fertility. In particular, extracts from *Chlorella vulgaris* and *Spirulina platensis* species have been reported to increase vitamin and antioxidant levels in agricultural crops (Bello *et al.,* 2021).

Studies in cereals show that microalgal biofertilizers optimise nutrient uptake and water use by promoting root and stem development. Furthermore, the capacity of microalgae to produce phytohormones improves crop quality by increasing photosynthesis and can increase overall crop yields by up to 20 (Ronga *et al*. 2019).

5.2. Biological Control

Microalgae can be used as biological control agents against plant pathogens. Species such as cyanobacteria play an important role, especially in the management of soil-borne diseases, by producing bioactive compounds with antifungal and antibacterial properties. For example, cyanobacteria such as *Anabaena* and *Nostoc* can suppress the growth of fungal pathogens by up to 70% by activating plant defence mechanisms (Costa *et al.*, 2019).

These bioactive compounds both increase the resistance of plants against abiotic stresses and reduce crop losses by inhibiting the proliferation of pathogens. Especially in crops such as rice, microalgae applications have been shown to reduce the impact of fungal pathogens while increasing crop yields (Ramakrishnan *et al*., 2023).

5.3. Use in Organic and Conventional Agriculture

Microalgal biofertilizers are becoming widespread as an environmentally friendly alternative in organic farming. These fertilizers can be used instead of chemical fertilizers, or they can be applied in an integrated manner with chemical fertilizers. The capacity of microalgae to increase organic matter is an important feature that supports soil health, especially in organic farming practices (Song *et al*., 2022).

The use of microalgae in conventional farming systems optimizes soil nutrient cycling and increases agricultural sustainability. This contributes to both the reduction of environmental impacts and the long-term preservation of agricultural production (Lu, 2022; Abu Ghosh, 2022).

These agricultural applications demonstrate both the economic and environmental benefits of using microalgae as biofertilizer and support the applicability of this technology in a wide range of agricultural areas.

6. Environmental and Economic Benefits

6.1. Reducing Chemical Fertilizer Dependency

Microalgal biofertilizers play a critical role in reducing negative impacts on the environment by replacing chemical fertilizers. By optimising nitrogen and phosphorus cycling, microalgae ensure that these nutrients are retained in the soil for longer, preventing groundwater pollution. For example, species such as *Chlorella vulgaris* store high levels of nitrogen and phosphorus, making them more efficiently available in the soil (Dagnaisser, 2022; Alvarez *et al.*, 2021; Solovchenko *et al*., 2016).

The overuse of chemical fertilizers not only causes environmental pollution but also threatens the sustainability of agriculture. The use of microalgal biofertilizers reduces this dependence in agricultural production, providing both economic and environmental benefits (Dagnaisser, 2022). As shown in Figure 2, microalgal biofertilizers provide numerous benefits, such as enhancing crop yield, reducing dependency on chemical fertilizers, and mitigating soil and water pollution.

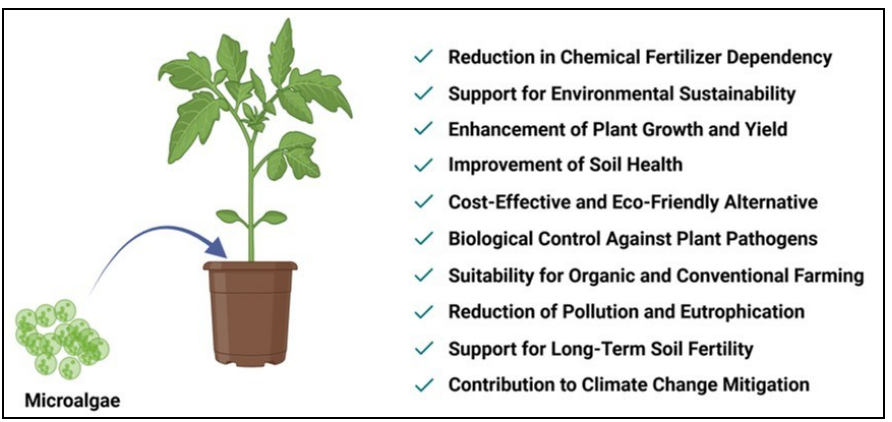

Figure 2: Benefits of microalgal biofertilizers in agriculture. Key advantages include enhanced crop yield and quality, reduced dependency on chemical fertilizers, mitigation of soil and water pollution, and promotion of environmental sustainability.

6.2. Reducing Soil and Water Pollution

Long-term and uncontrolled use of chemical fertilizers leads to eutrophication in water bodies, damaging aquatic ecosystems. Microalgae are an effective tool in solving this problem by biologically absorbing excess nutrients in soil and water. Species such as *Spirulina platensis* in particular can be used to reduce soil and water pollution. Microalgal biofertilizers also play an important role in combating climate change by reducing greenhouse gas emissions from agriculture (Renuka *et al*., 2018; Zhang, 2024; González *et al*., 2020).

Furthermore, life cycle analyses on the environmental impacts of microalgal fertilizers reveal that these products have significantly lower environmental impacts compared to chemical fertilizers. This supports the potential of microalgal fertilizers to promote environmental sustainability (Kumar and Singh, 2020).

6.3. Economic Feasibility

The economic viability of microalgal biofertilizers can be enhanced by production methods involving the use of wastewater and other biological wastes. Wastewaters are rich in nutrients such as nitrogen and phosphorus that promote microalgae growth, allowing microalgae to be cultivated at low cost (Mariyappan *et al*., 2024).

The commercial viability of microalgal fertilizers is particularly important in developing countries. These products provide a cost-effective solution for small-scale farmers while contributing to local economies. For example, studies in India have shown that the use of microalgal fertilizers can increase farm incomes by up to 15% (Sharma *et al*., 2021).

In addition, microalgal biofertilizers contribute to agricultural sustainability by recycling nutrients within a circular economy framework. This process benefits both economically and environmentally and offers a long-term replacement for chemical fertilizers (Lutzu *et al*., 2024).

7. Challenges and Constraints

7.1. High Production Costs and Scalability

Microalgae production requires high initial costs and operating expenses, especially when using technologies such as closed photobioreactor systems. Most of these costs are due to energy consumption, technical infrastructure and system complexity. For example, continuous control of temperature, light and nutrient parameters in closed systems increases operational costs. In addition, the energy intensity of harvesting and drying processes increases total production costs by 20-30 percent (Enamala *et al.,* 2018; Benner *et al.,* 2022).

Open-pond systems offer a low-cost alternative but face limitations such as contamination risk and low biomass yields. Scale-up studies are generally focused on increasing the capacity of bioreactors and optimising process efficiency. However, the need to maintain biomass yield and quality in these processes limits scalability (Tredici, 2009; Liu *et al*., 2019).

7.2. Stability and Shelf Life

The stability and shelf life of microalgal biofertilizers pose a significant challenge for commercial applications. Microalgal biomass is susceptible to problems such as water loss and microbial spoilage during storage. Low temperature storage and the use of specialised packaging materials are necessary to increase shelf life. For example, natural carriers such as paddy straw have been shown to be an effective method to extend the shelf life of microalgae biomass (Kumar *et al.,* 2020).

However, liquid formulations have been reported to have a longer shelf life, but in these formulations microbial populations may decline over time due to nutrient deficiency and oxygen deficiency. It is therefore recommended to apply biofertilizers quickly after their production (Lee *et al.,* 2016).

7.3. Regulatory and Certification Issues

Regulatory barriers need to be overcome for microalgal biofertilizers to be widely used in agricultural applications. Certification processes for these products can be time consuming and costly, especially for new formulations. In addition, these processes involve assessment of environmental impacts and product safety testing (Garbowski *et al.,* 2023).

The complexity of regulatory requirements is another important factor limiting the market penetration of microalgae-based products. Especially in developing countries, the lack of appropriate regulatory frameworks hinders the commercial viability of these products. In this context, government support and incentives are critical to accelerate the adoption of microalgal biofertilizers (Davis *et al*., 2014).

7.4. Environmental and Technical Constraints

Agricultural utilisation of microalgae can lead to environmental problems such as water and energy consumption. Especially in regions where water resources are limited, the applicability of these systems can be questioned. In addition, the risk of contamination is a serious problem, especially during production in open systems (Enamala *et al*., 2018).

Despite these challenges, innovative technologies and government policies can overcome these barriers. New formulation techniques to increase shelf-life, energy-efficient bioreactor systems and improved regulatory frameworks could contribute to a wider acceptance of microalgal biofertilizers in agricultural applications.

8. Future Perspectives

8.1. Innovative Technologies

The adoption of innovative technologies in the production processes of microalgal biofertilizers is critical to reduce costs and improve product quality. Genetic engineering enables the development of highly productive microalgae species, thereby

reducing production costs and increasing environmental sustainability. For example, genetic modification of cyanobacteria to increase their nitrogen fixation capacity enables them to be used more effectively in biofertilizers (Abinandan *et al.,* 2019).

Furthermore, the application of nanotechnology-based methods in microalgae production both optimises the release of nutrients and increases plant resistance. Nano-biofertilizers increase agricultural productivity and reduce environmental losses by providing controlled release of nutrients that support plant growth. These technologies make it possible to use microalgae, which are particularly sensitive to heavy metals and pesticides, in additional applications such as environmental clean-up and soil remediation (Miranda *et al*., 2024).

8.2. Policies and Incentives

To promote the widespread use of microalgal biofertilizers, it is necessary to develop policies that support sustainable agriculture. Government support can accelerate the adoption of this technology, especially in developing countries. The European Union's Green Deal strategy facilitates the integration of microalgal biofertilizers by providing a strong policy framework for sustainable agriculture and innovative Technologies.

However, national and international incentives can increase the adoption of microalgal fertilizers in agricultural applications. Incentives such as tax breaks, subsidies and research and development funds have been effective in increasing the economic viability of these products. For example, subsidy policies in India have encouraged small-scale farmers to use microalgal biofertilizers.

8.3. Integration with Other Biotechnological Solutions

Microalgae-based biofertilizers can be integrated with other biotechnological products such as biofuels and bioplastics. Such integration supports the circular economy in the agricultural sector and helps to achieve sustainable development goals. For example,

bioplastics produced from microalgae play an important role in waste management and carbon footprint reduction.

In addition, the use of microalgae in combination with environmental applications such as wastewater treatment increases the sustainability of this technology. This integration strategy both reduces costs and minimises environmental impacts in agricultural production. This versatile use of microalgal systems leads to lower carbon emissions and increased productivity in agriculture (Yang Ng, 2024).

Table 1: Key aspects of microalgal biofertilizers

Aspect	Details	Examples	References
Environmental Benefits	Reduces chemical fertilizer dependency.	*Chlorella vulgaris* reduces groundwater pollution.	Alvarez *et al*., 2021; Solovchenko *et al*., 2016
	Mitigates climate change through carbon sequestration.	Spirulina binds 180 tons of CO2 per hectare/year.	González *et al*., 2020; Renuka *et al*., 2018
	Decreases soil and water pollution by nutrient recovery.	*Spirulina platensis* in eutrophication control.	Kumar and Singh, 2020
Mechanisms of Action	Nitrogen fixation by cyanobacteria.	Anabaena, Nostoc.	Song *et al*., 2022; Alvarez *et al*., 2021
	Phosphorus solubilization through organic acid secretion.	*Cyanobacteria* increasing phosphorus availability.	Coppens *et al*., 2016; Alvarez *et al*., 2021
	Production of bioactive substances like phytohormones and polysaccharides.	Auxins and cytokinins in Chlorella vulgaris.	Lu *et al*., 2015; Song *et al*., 2022
Production Systems	Open ponds: low-cost but contamination risk.	Paddle-wheel mixed open systems.	Benemann ve Oswald, 1994; Kumar *et al*., 2020
	Closed photobioreactors: high yield but high cost.	Tubular and flat-plate photobioreactors.	Tredici *et al*., 2009; Borowitzka and Vonshak, 2017
	Hybrid systems: combines advantages of open and closed systems.	Initial sterile stage in bioreactors, final stage in ponds.	Liu *et al*., 2019
Nutrient Sources	Wastewater (rich in nitrogen and phosphorus).	Municipal and industrial effluents.	Acién *et al*., 2018; Wang *et al*., 2010
	Organic waste and agricultural residues.	Animal manure, compost extracts.	Das and Quadir, 2019
Applications	Enhances plant growth and yield.	Tomato, lettuce, and wheat studies.	Wuang *et al*., 2016; Chittora *et al*., 2020
	Biological control of pathogens through antifungal and antibacterial compounds.	*Anabaena* and *Nostoc* suppress fungal pathogens.	Ronga *et al*., 2019; Song *et al*., 2022
	Use in organic and conventional farming.	Replaces chemical fertilizers in organic systems.	Tiwari *et al*., 2019; Razzak *et al*., 2013
Challenges	High production costs and scalability issues.	Energy-intensive harvesting and drying processes.	Enamala *et al*., 2018; Benner *et al*., 2022
	Stability and shelflife concerns.	Microbial spoilage during storage.	Lee *et al*., 2016
	Regulatory and certification barriers.	Time-consuming product approval processes.	Garbowski *et al*., 2023
Future Directions	Integration with other biotechnologies (e.g., biofuels, bioplastics).	Circular economy in agriculture.	Lutzu *et al*., 2024
	Application of genetic engineering and nanotechnology to enhance productivity and sustainability.	Genetically modified cyanobacteria.	Mariyappan *et al*., 2024

Conclusion

Microalgal biofertilizers have significant potential for sustainability, environmental benefits and economic viability in the agricultural sector. Through mechanisms such as optimising nitrogen and phosphorus cycling, carbon sequestration capacities and phytohormone production, microalgae increase agricultural productivity while minimising environmental impacts. Their reduced dependence on chemical fertilizers and improved soil health makes these biofertilizers both an environmentally friendly and economical solution. However, solving current challenges such as production costs, scalability, stability and regulatory frameworks are critical for the widespread adoption of this technology.

In the future, the application of innovative technologies, the provision of appropriate incentives and integration with other biotechnological solutions will enable wider-scale use of microalgal biofertilizers. In particular, the integration of genetic engineering, nanotechnology and circular economy strategies in this field will increase the economic viability of this technology and support sustainable development goals in the agricultural sector. Microalgal biofertilizers will continue to play a critical role not only in agriculture but also as an integrated part of environmental and economic systems.

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CHAPTER VII

New record of Melanopsis costata (Olivier, 1804) in Kurtsuyu (Mersin, Türkiye): Morphological characters analysis using multivariate statistical methods

Filiz KUTLUYER KOCABAŞ[1]
Mehmet KOCABAŞ[2]

Introduction

Melanopsis snails belong to the subclass Prosobranchia of Gastropoda and commonly found in lakes, springs, and rivers across the Middle East and are widely distributed in the Mediterranean region. Despite this, there is limited information regarding the taxonomy of the genus and the evolutionary processes its populations have undergone. The shell structure of Melanopsis is soft, ribbed, elongated, grooved, banded, and entirely black,

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covering a small area. The genus is also known for its extreme variability (Gürlek et al., 2012; Kişin, 2019).

Geldiay and Bilgin (1969) identified mollusk species from various regions in Turkey such as Melaniidae, *Melanopsis costata, M. costata chantrei.* Bilgin (1980b) conducted a conchological comparison of *M. costata costata* and *M. costata chantrei* and concluded that there were no significant differences at the subspecies level. In a study, Bilgin (1986) compared *M. costata* with *M. preamorsa*, suggesting that *M. costata* might be classified as a subspecies. Ustaoğlu et al. (2003) examined the malacofauna of Yuvarlak Çay in Köyceğiz, Muğla, and documented the presence of M. costata from the Gastropoda class. Naser (2006) analyzed samples collected from southern Iraq to address uncertainties about the Melanopsidae family, reporting the existence of *M. costata, M. nodosa*, and *M. subtingitana*. Şereflişan et al. (2009) recorded *M. praemorsa ferussaci* and *M. costata costata*, along with other species from the Gastropoda class, while also detailing the physicochemical parameters of Gölbaşı Lake. Kişin (2019) reported M. costata in various water bodies, including Kuzgun Stream (Mersin), Burnaz Stream (Hatay), Gölbaşı Lake (Hatay), Asi River (Hatay), and Seyhan Dam Lake (Adana). This research provides the first scientific documentation of the Melanopsis costata (Olivier, 1804) population found in the Kurtsuyu (Mersin, Türkiye) along with an analysis of morphological characters using multivariate statistical methods

Material and Methods

The *M. costata* used in this study were collected from the Kurtsuyu (coordinates: 36,510158, 33,543725 North/East) during July 2024 (Figure 1).

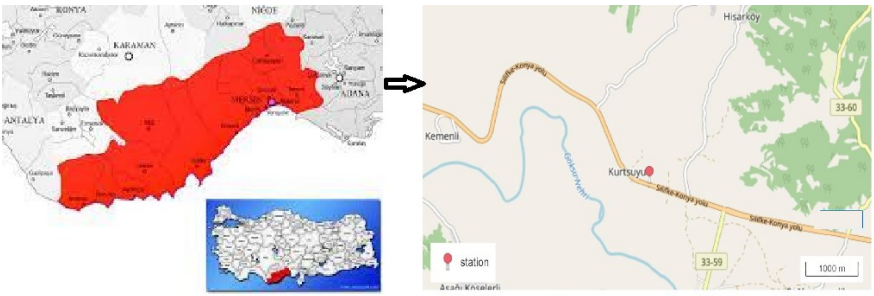


Figure 1: The map of sampling area.

Samples were collected using metal-framed scoops, shovels, and a rake for dredging the bottom. The snails were gathered by hand from sandy and natural areas at depths of up to 1 meter and stored in styrofoam boxes at +4°C. Identification of the snails was carried out by comparing them with the descriptions and illustrations of different *Melanopsis* species found in the literature (Glöer, 2002, 2019; Kruglov, 2005; Glöer & Girod, 2013; Gürlek, 2015) (Figure 2).

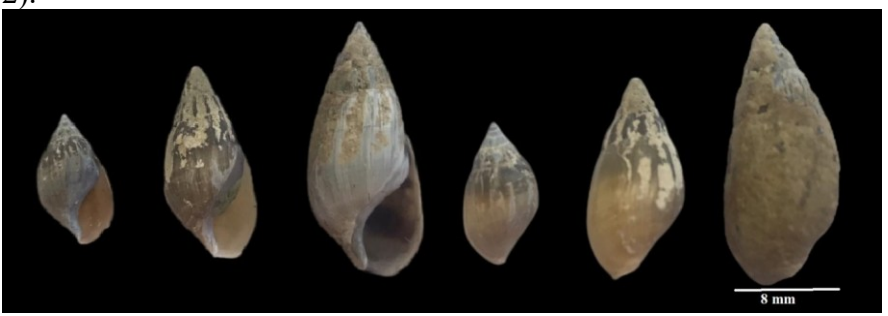


Figure 2. Melanopsis costata collected from Kurtsuyu.

The shell measurements, such as shell length, shell width, aperture length and width, spire height, and body whorl height, were obtained using a digital caliper with an accuracy of ±0.01 mm. The weight of the specimens was measured using a precision scale with a sensitivity of ±0.001 g (Figure 3).

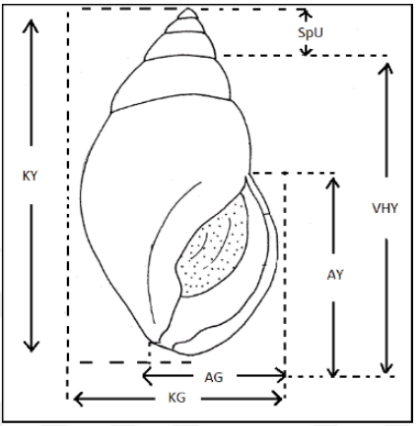


Figure 3: Morphometric measurement in Melanopsis costata (Gürlek et al., 2012).

To assess the relationships between biometric parameters, correlation analysis was conducted, with data analysis and processing carried out using Microsoft Excel®. Principal component analysis (PCA) was performed using the Past 4.03 software to explore the interactions among the variables.

Results and Discussion

This study marks the first report of *M. costata* in Kurtsuyu (Mersin, Türkiye). Due to phenotypic variations in shell morphology, identifying species can be quite challenging in many taxa, and the resemblance in shell characteristics often leads to the overlooking of potential new species in research. Understanding the malacofauna of Kurtsuyu is crucial for gaining insights into the freshwater snail fauna and zoogeographic distribution of Turkey.

In the current study, the mean measurements for shell length, shell width, aperture length and width, spire height, body whorl height, and weight of Kurtsuyu were 17.98±4.03 mm, 7.78±1.30 mm, 8.68±1.47 mm, 5.52±1.21 mm, 5.28±1.72 mm, and 0.69±0.33 g, respectively. Kişin et al. (2019) reported the largest shell length and width as 23.7 mm and 11.6 mm from Osmaniye Hemile Bridge while the smallest measurements were 12.3 mm and 6.6 mm from

Adana Seyhan Dam. In this study, the maximum values for shell length (SL) and weight (W) were determined as 22.64 mm and 1.056 g, respectively.

Principal Component Analysis (PCA) revealed a notable association among shell width (SW), spire height (SPH) and weight (W) (Figure 4). In gastropods, these morphometric features often show correlated variations, which may be influenced by biological and ecological factors that affect shell growth and body weight (Atıl et al., 2024).

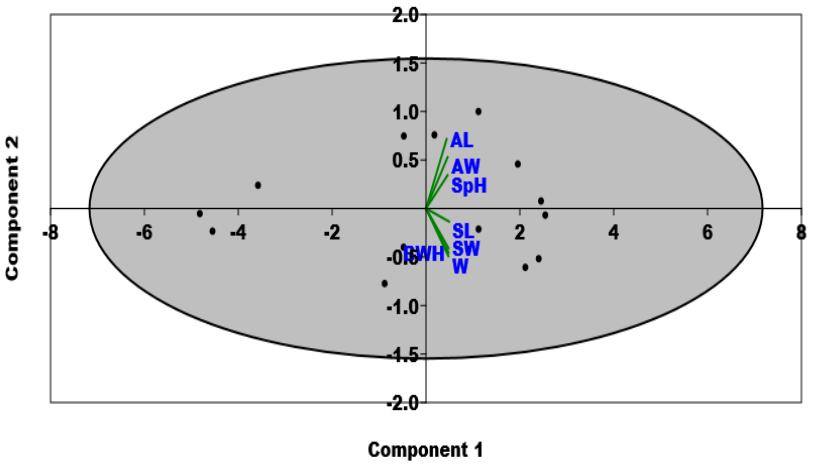
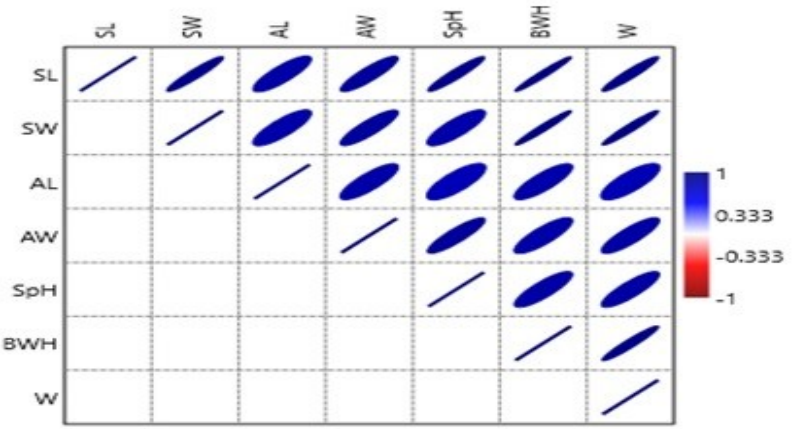


Figure 4: Biplot of the PCA (Principal Component Analysis) illustrating the correlation between shell characteristics (SL, SW, AL, AW, SPH) and weight (W) of M. costata.

This study revealed a strong correlation between shell length and shell width, shell length and aperture length, shell length and body whorl height, shell length and weight, shell width and aperture length, shell width and body whorl height, shell width and weight, aperture length and body whorl height, and aperture length and weight in Kurtsuyu (Figure 5).



	SL	SW	AL	AW	SpH	BWH	W
SL							
SW	0,9477						
AL	0,83846	0,82535					
AW	0,88635	0,87075	0,8287				
SpH	0,94472	0,82307	0,77881	0,88406			
BWH	0,97379	0,97295	0,8257	0,83072	0,84539		
W	0,95038	0,96465	0,78611	0,85012	0,85424	0,95572	

Figure 5: Correlation matrix of the shell properties (SL, SW, AL, AW, SPH) and weight (W) of M. costata in Kurtsuyu.

Consequently, Kurtsuyu is newly identified localities for M. costata in Turkey. The observed morphological variations between different sampling stations are likely influenced by factors such as diet, water chemistry, habitat conditions, and water temperature. Given the high potential for subspeciation or even speciation in Anatolia, a region known for its significant molluscan diversity and endemism, additional studies on the molecular taxonomy of the specimens will further support our findings. These studies will also clarify the status of the genus in Turkey and help elucidate the factors driving morphological differentiation.

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CHAPTER VIII

Evaluation of sperm quality parameters of sex-reversed rainbow trout (*Oncorhynchus mykiss*) in a commercial farm in Black Sea Region using multivariate statistical methods

Filiz KUTLUYER KOCABAŞ[1]
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Introduction

In Türkiye, aquaculture production has become an important sector that provides employment and develops for the last twenty years. The most commonly farmed rainbow trout (*Oncorhynchus mykiss*) was first produced in America in 1874, using broodstock caught from the McCloud River in Northern California, under artificial conditions in a special hatchery in Caledonia (New York) (Gall, 1974). It was first grown in a marine environment in Norway

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in 1912 (Edward, 1978). Its commercial production became widespread mainly in the 1930s. Today, it is grown in more than 100 countries. Many breeding studies have been carried out on rainbow trout and more than 15 commercial varieties have been obtained (Vandeputte et al., 2008).

In parallel with the progress in the world, there have been significant developments in the aquaculture sector in Turkey, and a rapid development was achieved with the culture of rainbow trout in net cages in inland water ponds in the early 1980s and in sea cages in the 1990s (Akbulut et al., 2009). The rapid increase in production has created some problems. With the impact of the support given to the sector, private sector investments, which have a dynamic structure, have increased, and the need for fry has emerged as the production in net cages in the dam lakes has increased more than expected. Demand for fry from the region encouraged the increase of existing hatchery capacities, and in the first place, fry fish needs were tried to be met by transporting live fish from across the country (Salihoğlu et al., 2013).

Obtaining the desired results in aquaculture depends on being able to control the entire production cycle of the species being grown, ensuring that the parent individuals have a good genetic structure, effectively controlling diseases and preventing contamination, providing quality and sufficient water to the environment, the application of innovative management techniques (Okumuş, 2002). Sperm quality is vital for aquatic organisms, as it directly impacts success of fertilization rates and hatching in aquaculture operations (Kutluyer et al., 2017; Kocabaş et al. 2017a,b,c). Decreased sperm quality can lead to extinction or loss of populations. The current study is aimed to investigate of sperm quality parameters of sex-reversed rainbow trout (*Oncorhynchus mykiss*) in a commercial farm in Black Sea Region using multivariate statistical methods.

Material and Methods

Masculinization and sample collection

Masculinization of sex-reversed females was carried out at the Ayta Production Facility (Rize, Türkiye) using 17α-methyltestosterone (MT). These females were administered MT through their feed at a concentration of 2 mg/kg, for a duration of 60 days at a temperature of 10°C. After the experiment, milt was extracted from these individuals by dissecting the testes and gently pressing them through double-layered gauze to eliminate any residual testicular tissue.

Analysis of sperm quality parameters

A phase-contrast microscope (Nikon CI, Tokyo, Japan) with 200X magnification, captured via a CCD camera (Nikon DS-Fi, Nikon, Japan) with computer-assisted sperm analysis (SCA) was used for evaluation progressive motility. Fresh sperm samples (n:20) were activated using a 0.3% NaCl solution. The percentage of motile sperm was determined based on actively moving sperm, and the duration of forward motility was recorded as the time between sperm activation and cessation of movement. For the experiment, fresh pooled sperm with normal pH, volume, and motility above 70% was used. Spermatocrit was calculated according to Rurangwa et al. (2004) and expressed as a percentage, calculated by the ratio of the packed white material volume to the total semen volume × 100.

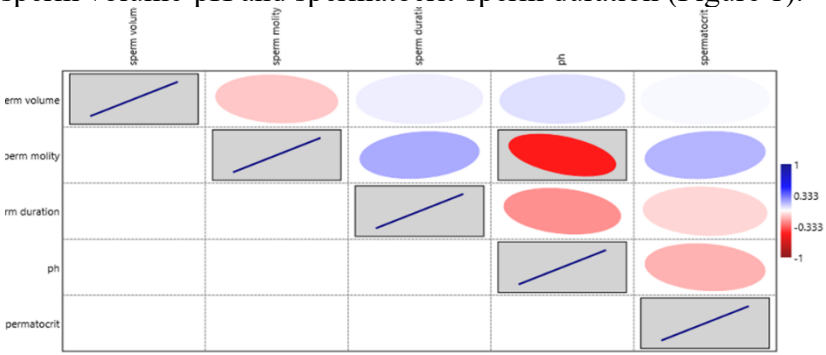
Data analyses

Correlation analysis and Principal component analysis (PCA) were conducted using PAST4.03 software to explore the relationships between variables. A significance level of $p < 0.05$ was set for the analysis.

Results

Sperm volume, motility rates, duration, pH and spermatocrit of fresh sperm for sex-reversed rainbow trout were 5.30±2.35, 91.59±5.65%, 27.14±6.92 s, 7.38±0.22, 51.50±12.51, respectively.

In this study, it was determined that there is a strong correlation between sperm volume-spermatocrit, sperm volume-sperm duration, sperm volume-pH and spermatocrit-sperm duration (Figure 1).



	sperm volume	sperm molity	sperm duration	ph	spermatocrit
sperm volume					
sperm molity	0,6293				
sperm duration	0,88555	0,46331			
ph	0,78205	0,036094	0,33178		
spermatocrit	0,9529	0,51484	0,72312	0,50659	

Figure 1: Correlation matrix of the sperm quality parameters of sex-reversed rainbow trout (Oncorhynchus mykiss) in a commercial farm in Black Sea Region.

In this study, KMO and Bartlett's test data, which are useful in summarizing the correlation matrix, were determined to be significant ($p = 0.000$; $p < .001$). Therefore, principal components analysis has been found to be useful and the variables are interrelated. pH has negative scores featuring the sperm (Figure 2).

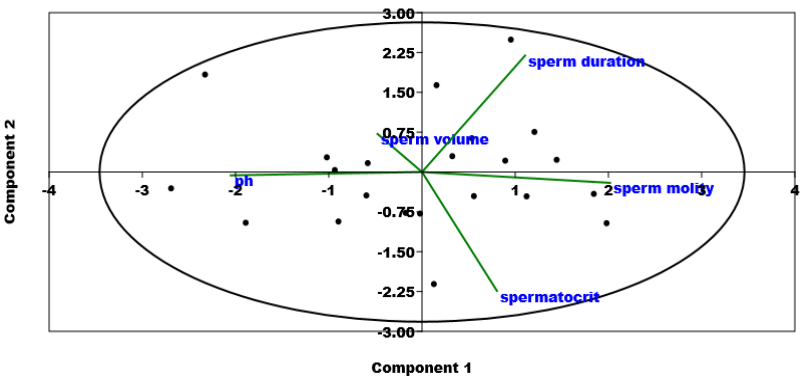


Figure 2: Biplot of Principal Component Analysis (PCA) analysis of variables (Sperm volume, motility rates, duration, pH and spermatocrit).

Discussion

In trout, males generally reach sexual maturity earlier than females (Büyükhatipoğlu and Holtz, 1984; Billard, 1995). During the breeding season, male fish are triggered by gonadotropins to prepare for sperm release. The sperm cells remain inactive within the testes but become motile upon exposure to water in controlled (*in vitro*) conditions. The motility of sperm in water is short-lived, lasting anywhere from 30 seconds to 2 minutes, depending on the water temperature. Sperm production in most fish of this family is exceptionally high (5×10^{12} spermatozoa/kg body weight). However, under natural conditions, only about 20-40% of the semen produced is utilized with the excess being reabsorbed by the body (Aegerter et al., 2005). The sperm quantity in fish from the Salmonidae family differs across species. Additionally, variations can occur among individuals of the same sex. Not only is there variation in the amount of semen, but also in other qualitative characteristics at different stages of the breeding season—beginning, middle, and end. In rainbow trout, the average semen volume ranges

from 6 to 12 ml, although this amount can be influenced by various factors. Key factors reported to significantly affect semen production include water temperature, the interval between semen stripping, age, and particularly the conditions related to care and feeding (Büyükhatipoğlu and Holtz, 1984). The variation in sperm volume in rainbow trout is considerably wide. In this study, sperm volume of fresh sperm for sex-reversed rainbow trout were 5.30±2.35. As a result of stripping, males give sperm; masculinized females will not be able to produce sperm because the testes are abnormal and the excretory ducts are absent. To obtain sperm from these, the secondarily developing testicles are removed by opening the abdomen with the help of a scalpel. A small sample of semen fluid is collected from the testicle and examined under a microscope. After assessing the sperm's viability and density, it is then used to fertilize the egg (Akhan and Canyurt, 2005; Emre and Kürüm, 2007).

Sperm motility and duration are crucial for the continuation of the species, as they influence success of fertilization and hatching (Öğretmen et al., 2016). In Salmonids, the release of sperm cells into the water is essential for the initiation of sperm movement (Ingermann et al., 2002; Dzyuba et al., 2010; Öğretmen et al., 2016). The composition and concentration of the activation medium are key factors in the initiation and progression of sperm motility. In present study, motility rates and duration of fresh sperm for sex-reversed rainbow trout were 91.59±5.65% and 27.14±6.92 s, respectively.

The pH value of the semen is determined with indicator papers or pH measuring instruments in fresh semen and after the dilution process. The measurement technique used during pH measurement must be strictly followed. Changes in pH value detected in semen may indicate that any external substance is mixed into the semen. In addition, waste substances (such as lactic acid) released by spermatozoa into the environment as a result of metabolic activities can also cause pH changes. In scientific studies and in the use of semen for various purposes, the change in pH value within a certain period of time depending on spermatozoon metabolism can be used as a criterion in the evaluation of semen (Büyükhatipoğlu et al.,

1984; Munkıttrıck et al., 1987). In current study, the mean of pH in fresh sperm for sex-reversed rainbow trout was 7.38±0.22.

Semen usually varies in color from light cream to dark cream. The number of spermatozoa in the semen has a lot to do with the color formation. Except for pathological conditions, normal differences can be observed in the color of semen depending on the animal species and nutrition. Abnormal colors may also be encountered in semen. These situations are signs that there is an abnormal condition in the genital organs or foreign substances are mixed into the semen (Kutluyer and Kocabaş, 2017). In this study, the color of semen was white.

In conclusion, the increase in world population, technological developments, environmental pollution and ecological problems causes a decrease in animal protein resources. These problems have made it necessary to obtain more animal protein per unit area. At this point, aquaculture comes to the fore. Studies aimed at increasing productivity in cultivation have also gained importance in recent years. Biotechnological studies carried out in this direction are of great importance. Data on broodstock management of rainbow trout farmed in Türkiye is extremely scarce. However, quality fry production can be done by hatcheries whose broodstocks have superior characteristics. This is possible by primarily revealing the characteristics of the broodstock used in production. Because the fertility of trout broodstock is under the influence of many factors. Similar studies to be carried out in different regions will make significant contributions to the production of quality broodstocks.

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CHAPTER IX

Is the garlic-lemon cure beneficial for testicular tissue?

Gulsah YILDIZ DENIZ[1]

Introduction

Acetic acid bacteria (AAB) have, for centuries, been important microorganisms in the production of fermented foods and beverages such as vinegar, kombucha, (water) kefir, and lambic beer (Raspor & Goranovič, 2008). Their unique form of metabolism, known as "oxidative" fermentation, mediates the transformation of a variety of substrates into products, which are of importance in the food and beverage industry and beyond; the most well-known of which is the oxidation of ethanol into acetic acid (Guillamón & Mas, 2017). AAB, first described as “vinegar bacteria” by Louis Pasteur over 150 years ago, are an important and diverse group of bacteria involved in the production of fermented foods and beverages,

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especially known for their production of acetic acid (ethanoic acid) in the making of vinegar. (Pasteur, 1864). AAB are associated with, and have been isolated from, carbohydrate-rich and acidic environments such as fruits and flowers, and are involved in the production of a variety of fermented foods and beverages including vinegar, kombucha, lambic beers, kefir, and nata de coco (Gomes et al., 2018).

Lemon juice behaves as a natural ionic electrolyte. The main ingredients of this extract are moisture (85%), citric acid (5–7%), carbohydrates (11.1%), vitamin C (0.4%), protein (1%), fat (0.9%), minerals (0.3%), fibers (1.7%), and some free and combined organic acids. As lemon juice is acidic in nature (pH about 2–3) and the percentage of citric acid is 5–7%, it will be worked as acid catalyst for condensation. Citrus lemon (Citrus limonBurm.F) is a source of vitamin C, flavonoids and carotenoids (González-Molina et al., 2010). Eriocitrin and hesperidin are the main flavonoids in lemon. The antioxidant activity of eriocitrin is more potent than other citrus flavonoids (Miyake et al., 2007).

Garlic acquired a reputation in many cultures over centuries as a prophylactic and therapeutic medicinal agent. In the past decade, some protective effects of garlic have been well established by epidemiological studies and animal experiments (Kooket al., 2009; Ansaryet al., 2020). Commercially available garlic preparations in the form of garlic oil, garlic powder, and pills are widely used for certain therapeutic purposes, including lowering blood pressure and improving lipid profile.

The therapeutic effects of garlic and lemon have been discussed in many articles, but the two have never been studied together (Percival, 2016; Jana & Pradhan, 2021). The garlic and lemon cure has become a well-known drink among the public and is especially recommended for blood pressure patients (Ried & Fakler, 2014; Wang et al., 2015). However, the fact that no research has been done on this cure so, it makes people nervous about its use.

Cadmium (Cd) is a poisonous environmental pollutant and can induce oxidative stress and classified as a human carcinogen (Nordberg et al., 2018). Cd is widely used in batteries, plastics, colors, and electroplated (Elinder, 2019). The main way for smokers or professional workers to ingest cadmium is through inhalation. Industrial workers as well as people in general, are exposed to cadmium given its increased production and usage (Sarkar et al., 2013). Cadmium can induce various severe pathological conditions such as hepatic and renal dysfunctions, testicular harm, and nervous system issue (Gobe & Crane, 2010).

The aim of this study is to investigate the possible therapeutic properties on Cd induced testicular damage of the new fermented product formed as a result of the co-fermentation of these two important foods.

Materials and Methods

Animal preparation

In this study, 28 male Wistar Albino rats weighing 230–250 g were used. The rats were obtained from Ataturk University

Experimental Research and Application Center. Animals were housed in standard cages under well-regulated conditions (relative humidity range: 45 ± 5%, temperature: 24 ± 1°C and a 12-h light/12-h dark cycle). During the experiment, rats were fed with standard rat diet and water ad libitum.

Experimental design

Wistar albino rats were randomly divided into four groups, including one control and the following three experimental groups: a Cd group (0.025 mmol/kg), a GLC group (15 mg/kg/day orally for 5 days), and a GLC + Cd group (15 mg/kg/day orally for 5 days and Cd 0.025 mmol/kg by intraperitoneal injection on the fifth day).

Euthanasia

The rats were euthanised 5 days after the therapy. Prior to euthanasia, the rats received anaesthesia using a combination of ketamine and xylazine. Euthanasia was performed by decapitation.

Preparation of tissue homogenates

The tissue samples from each rat were first perfused with PBS/heparin and then ground in liquid nitrogen using the TissueLyser II grinding Jar Set (Qiagen, Hilden, Germany). Approximately 100 mg of ground tissue was homogenized in 1 ml PBS homogenate buffer in an eppendorf tube with TissueLyser II, and the samples were then centrifuged.

Biochemical analysis

Lipid peroxidation, a measure of free radical damage, was assayed by MDA. Biochemical assays: The lipid peroxidation (LP) content was exhibited by the MDA level with thiobarbituric acid reaction. The animal testicles were transferred to −80 °C. Thereafter, testis samples were homogenized into phosphate-buffered saline (PBS) and centrifuged at 10,000g, 4 °C for 10 min. The supernatants (40 µl) were collacted and immediately transferred to −80 °C for further determination. Enzyme linked immunosorbent assays (ELISA) kits were used to measure the levels of SOD and MDA according to the manufacturer's instructions.

Histopathology

The testicular tissues were fixed in 10% neutral buffered formalin for 48 h, dehydrated with different concentrations of ethanol, cleared with xylene and embedded in paraffin. Then, 4 µm-thick sections were prepared and stained with hematoxylin and eosin. The structural changes of the sections were assessed by optical microscopy.

Statistical analysis

The results were expressed as mean± standard error. The statistical significance between the different groups was determined using one-way analysis of variance (ANOVA) with GraphPad Prism 5.0 statistics software (GraphPad, La Jolla, CA, USA). Tukey's test was carried out for betweengroup comparisons using the Tukey multiple comparison test. Analyses between two groups were carried out using the Mann–Whitney U test. Statistical significance was set at $P<0.05$.

Results

No animal died throughout the course of the experiment. GSH were assayed in testis homogenates from control and experiment groups.

MDA Assay

The GLC treatment promoted a significant reduction ($P < 0.001$) in testicular concentrations of malondialdehyde compared with Cd group (Figure 1)

GSH Assay

The pretreatment with GLC promoted a significant increase ($P < 0.001$) in GSH testes contents compared with Cd pretreated rats (Figure 2).

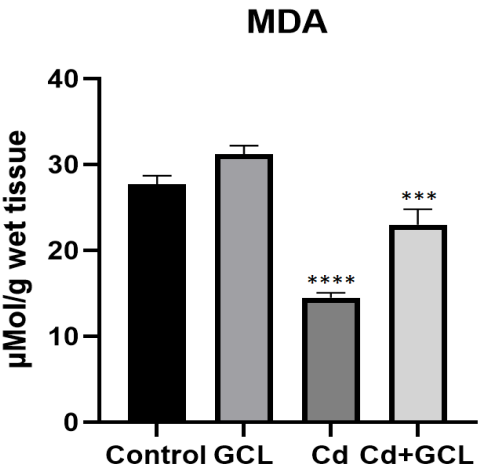

Figure 1. *Data are presented as mean ± SEM (n=5). a denotes significant differences between other studied groups and control (a1:p<0.05, a2:p<0.01, a4<p.0001), b denotes significant differences between other studied groups and RIR group (b2:p<0.01, b4:p<0.0001) by Tukey's multiple range tests.*

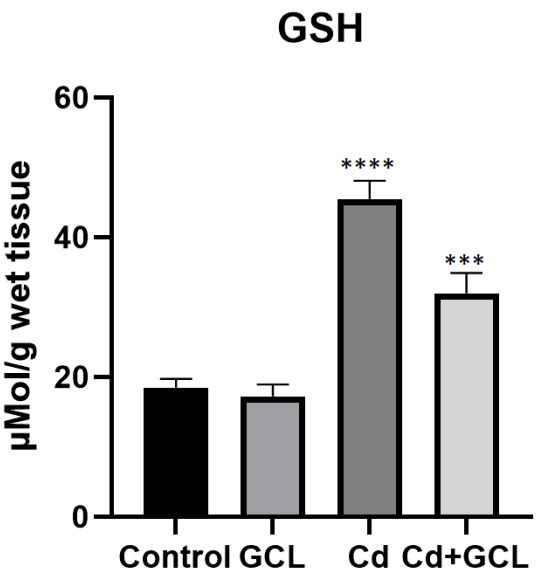

Figure 2. *Data are presented as mean ± SEM (n=5). a denotes significant differences between other studied groups and control (a1:p<0.05, a2:p<0.01, a4<p.0001), b denotes significant differences between other studied groups and RIR group (b2:p<0.01, b4:p<0.0001) by Tukey's multiple range tests.*

Histological assessment of seminiferous tubules

The testicular seminiferous tubules of rats in the control group (Fig. 3A) and the GLC-treated group (Fig. 3B) were arranged in order, interstitial clear, seminiferous tubules seen in all stages of spermatogenic cells. Examination of tissue sections affirmed the extreme morphological changes in the testes of cadmium-treated rats. In cadmium-treated rats, the spermatogenic cells in the testicular seminiferous tubule showed disordered arrangement and structure, and their levels were unclear; meanwhile, the sperm chromatin structure of the testes in the cavity of the spermatogenic small tube could not be observed (Fig. 3C and D). In the Cd+GLC

group (Fig. 3D), rat testicular tissue structure were significantly improved, and germ cells levels of in the tubule of rats were basically in order.

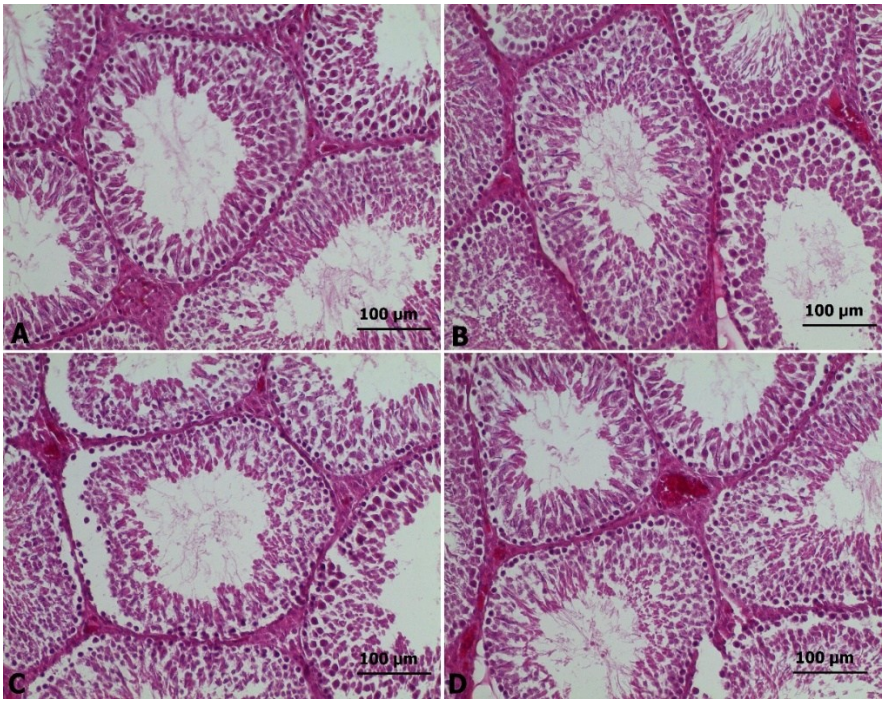

Figure 3. Testicular histopathology of rats treated with cadmium and GLC (B, C,D 100×); A: control showing seminiferous tubules arranged in order, interstitial clear, seminiferous tubules seen in all stages of spermatogenic cells; C, D: cadmium treated rats showing the spermatogenic cells in the testicular seminiferous tubule showed disordered arrangement and structure, and their levelswere unclear; meanwhile, the sperm chromatin structure of the testes in the cavity of the spermatogenic small tube could not be observed; B: GLC alone treated rats showing normal appearance of testicular structure; D: cadmium and GLC treated showing germ cells levels of in the tubule of the rats were basically in order.

Discussion

Cadmium is a toxic metal, which promotes oxidative stress and contributes to the development of serious degenerative changing in several tissues (Sharma et al., 2015). The effects of cadmium have been shown to be due to oxidative damage by enhancing the peroxidation of membrane lipids in different tissues (Jawad et al., 2020). It is well known that the testis is very sensitive to cadmium toxicity. It was reported that the testis could be protected from the toxic effects of cadmium, mainly by antioxidant treatment. Garlic has been used throughout history for both culinary and medicinal purposes. A. sativum is a versatile herb that contains numerous vitamins, minerals and trace elements (Capasso, 2013). Citrus fruits and juices are rich sources of bioactive compounds, like flavonoids, carotenoids, limonoids, coumarin-related compounds, folates, essential oils, pectins and vitamin C (Saini et al., 2022). Vitamin C, known as ascorbic acid, acts as a powerful antioxidant and may reduce the risk of cardiovascular diseases, arteriosclerosis, and some forms of cancer (Al-Khudairyet al., 2017; Blaszczak et al., 2019).

Our results indicate the effects of Cd on the male rat testis tissue. The male rat testes tissue MDA levels increased significantly by Cd treatment. The results suggest that the increased level of MDA, which resulting from testicular damage, affected testicular spermatogenesis.

The effect of Cd on testicular function in male rats was severe with lowered activity of GSH. In the present study, it was determined that GLC showed a protective effect on the reproductive system of male rats that were exposed to Cd.

It is well known that Cd has various carcinogenic effects on internal organs such as the liver, kidneys and lungs, and a toxic effect on the immune system, genes, blood, nervous system, and reproductive system (Andjelkovicet al., 2019; Mirkov et al., 2021).

In the present study, when the testes of rats treated with Cd and GLC were examined; In the control, seminiferous tubules were regularly arranged, interstitial clear, and spermatogenic cells were seen in all stages of seminiferous tubules (Figure 3A and 3B, respectively). In Cd-treated rats, spermatogenic cells in the testicular seminiferous tubule showed irregular arrangement and structure, and their levels were unclear (Figure 3C). Germ cell levels in the tubules of rats treated with Cd and GLC were basically regular, and the image was similar to the control (Figure 3D). Several studies have shown the harmful effects of Cd on testicular tissue (Bhardwaj et al., 2021; Ali te al., 2022).

As a result of all this information, it was concluded that GLC may have a protective effect on Cd-induced testicular damage.

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CHAPTER X

Bacterial Cellulose Production and Applications in Cosmetics

Murat ÖZDAL[1]
İrem ÇELİK[2]

Introduction

Cellulose is a naturally occurring biopolymer found abundantly on Earth. It is commonly obtained from plants, but is also obtained from certain bacteria (Choi et al., 2022). Bacterial cellulose (BC) produces *Acetobacter xylinus* was first recorded by Brown in 1986 (Lupaşcu et al., 2022; Almihyawi et al., 2024). The differences between plant and bacterial cellulose are due to the supramolecular structure, biosynthesis pathways, purity, crystal structure and degree of polymerization (Rosson et al., 2024). Although plant and BC share the same molecular formula ($C_6H_{10}O_5$), BC does not contain the lignin, hemicelluloses, or pectin that are commonly present in celluloses derived from plants. Consequently, BC purification is an easy and low-energy procedure (Azmi et al., 2023). In Figure 1, the

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molecular structure is displayed. Cellulose, which is a type of polysaccharide, be made up of β(1-4)-linked D-glucose units. Each glucose subunit binds to nearby glucose monomers in the chain to create cellulose microfibrils using hydrogen bonds and van der walls forces (Rongpipi et al., 2019; Choi & Shin, 2020).

Cellulose is an unbranched-linear polymer containing glucose repeating component linked together by β(1-4) glycosidic bonds. Cellobiose is formed when the OH groups at C-1 and C-4 of glucose join with ether bonds during dehydration (Heinze, 2016). The cellulose molecular chain contains numerous polar hydroxyl groups. The C-6 position is the major OH group, whereas the C-2 and C-3 positions are subsidiary OH groups (Zugenmaier, 2008).

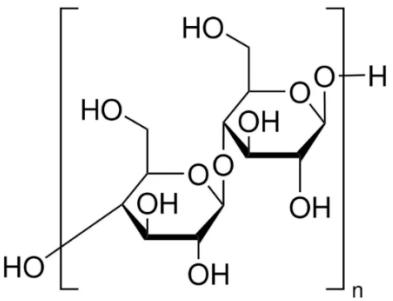

Figure 1. Chemical structure of cellulose

Bacterial celluloses have wide-scale applications (textile, paper, biomedical, cosmetic, energy, food, environment etc.) due to their exceptional purity, crystallinity, and water retention capacity (Pandit & Kumar, 2021). BCs can also be used to create a variety of functional materials, including carbon dots, porous membranes, and soft hydrogels. Cellulosic materials are frequently employed in wound dressing, food packaging artificial blood vessels, tissue engineering, and biosensors (Cheng et al., 2023). Considering the current research, much progress has been made in the research on the use of cellulosic materials in cosmetic applications. However, the

mechanism of action between molecular structure arrangement, material morphology, structure, and performance needs to be studied in greater detail (Cheng et al., 2023). In this section, we explain the production of BC and their different uses in the cosmetic industry.

Factors Affecting Bacterial Cellulose Production

The most well-known and prolific bacterium is *Gluconacetobacter xylinum*, an aerobic Gram-negative bacterium that thrives in environments high in sugar and can with stand up to 10% of glucose (Li et al., 2022). Other bacterial groups used in BC production are Salmonella, Acrohomacbacter, Aerobacter, Agrobacterium, Gluconabacter (Acetobacter), Psuedomonas, Rhizobium, Sarcina, and Komagatiabacter (Picheth et al., 2017; Gregory et al., 2021; Mbituyimana et al., 2021; Choi et al., 2022).

In general, the produced BC consist of a transparent gel-like film made of interlocking cellulose microfibrils of undetermined length scattered at random orientation (Picheth et al., 2017).

Dissolved oxygen, pH, temperature, time, inoculum concentration, static or shaking production methods are important factors in BC production (Lee, 2014). These factors also affect the crystal polymorphology, crystallinity index and porosity of the produced cellulose (Yan, 2008).

Temperature is one of the most important fermentation conditions and affects the normal metabolism of the organism. The most suitable temperature range for BC production is between 25-30 ℃. While Komagateibacter provides maximum efficiency in a static environment for 7 days at 30 ℃, the suitable temperature for BC produced by *Aceobacter xylinus* is 28 ℃ (Wang, 2018; Son, 2001; Zahan, 2017). The preferred temperature range for BC synthesized by Gluconabacter sp. RV28, Pseudomanas sp. RV14, Enterobacter sp. RV11 is between 28-30 ℃. Production efficiency decreases at high temperatures, while microbial growth decreases at low temperatures (Ranaswamy, 2015).

pH is an important parameter in the oxidative fermentation of BC. Acidic or near-neutral pH is ideal for BC synthesis. Secondary metabolites such as lactic acid, acetic acid, and gluconic acid are also produced during BC fermentation, which can change the pH of the fermentation culture medium. Experimental findings show that BC production is at pH 5.5 for *Acetobacter xylinus* (Zakaria, 2012), 4.5–7.5 for *Acetobacter xylinus* (Son, 2001), and pH 6 for Komagotaebacter (Wang, 2018).

The composition and properties of bacterial cellulose are affected by the production method (fixed or shaken cultures) (Czaja et al., 2004; Wang et al., 2019). The conventional technique of static culture can create a BC film or membrane with superior structure and qualities at the air-liquid interface. The thickness of BC is typically closely correlated with the duration of incubation (Wang et al., 2019). The area of the air culture medium interface limits BC productions in static cultures because oxygen consumption is proportionate to BC output. In shaking cultures, the medium can potentially offer adequate aeration, hence speeding up BC production and increasing efficiency (Gregory et al., 2021). In some culture conditions where bioreactors are used, contacting the liquid with air or increasing bacterial concentrations and changing the equipment used in production may help increase BC production.

One of the most important factors affecting the formation of BC is the shaking speed. At a shaking speed of less than 100 rpm, 0.5 - 1 cm of regular solid sphere cellulose is formed. Between 150 - 250 rpm, the BC size reduces at 300 rpm, the cellulose aggregates. Shaking speed affects the shape of the BC (Lahiri et al., 2021). Since the bacteria in the culture media are aerobic, they require sufficient oxygen supply. Low levels of oxygen inhibit BC production (Lahiri et al., 2021).

In BC production, the nutritional content of the production environment is as important as the environmental conditions. In general, in studies, the effects of carbon (glucose, sucrose, fructose, glycerol), nitrogen (yeast extract, peptone, ammonium acetate,

ammonium sulphate), and other components (citric acid, KH_2PO_4, Na_2PO_4, $MgSO_4$, and $CaCl_2$) are investigated by taking the HS environment into account (Shoda & Sugano, 2005; Aswini et al., 2020; Sperotto et al., 2021).

BC is an important biomaterial for various applications. However, its production on an industrial scale is a challenge due to the high production media cost and low efficiency. To overcome this problem, low-cost substrates and waste by-products of various industries are used for BC production. For this purpose, wastes from food, agriculture, textile, beer, and sugar industries are ideal substrates for BC production. Researchers have evaluated the wastes of these industries for low-cost BC production (Hussain et al., 2019; Abol-Fotouh et al., 2020; Güzel & Akpınar, 2020).

Properties of Bacterial Cellulose

The porous structure of BC makes it suitable for use in many areas. Due to this structure, it has high water retention properties and is very difficult to disperse in water (Torres et al., 2012). For this reason, this polymer is especially desired in cosmetics in terms of keeping the skin moist. The fact that BC is non-toxic, biodegradable, has water retention properties, is easy to shape, and is light provides advantages over other polymers (Bäckdahl et al., 2006; Popa et al., 2022). It allows chemical changes due to the empty hydroxyl groups (McNamara et al., 2015). Since it is very functional, it has many different areas of use such as optics, electricity, food, environment, and medicine (Gregory et al., 2021). In addition, its other advantages include the fact that it can be obtained in high purity and produced from renewable resources (Figure 2).

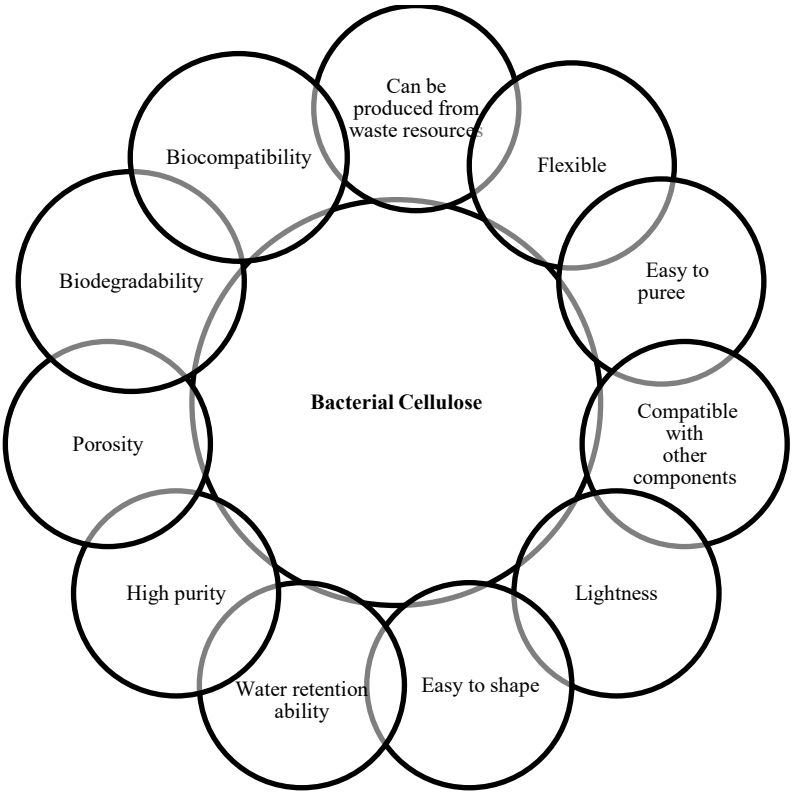

Figure 2. Properties of bacterial cellulose

Cosmetic Applications of Bacterial Cellulose

As a result of the changing and increasing perception of beauty in the world, cosmetic products are being developed and applied to the skin to support healthy living. Natural sources such as BC hydrogels and nanofibers with different structures have been used as basic ingredients to develop cosmetic products. The average diameter of the three-dimensional nanofiber network known as BC is 70 nm (Tang et al., 2010), and the porosity can be increased by different pretreatments (Munir et al., 2024). Furthermore, BC can be loaded with various nutrients and even components with therapeutic functions due to its highly porous microstructure (Chantereau et al.,

2020). This BC property has been used by many facial mask manufacturing companies in the cosmetic industry. Cosmetologists have been able to release hydrophilic and hydrophobic cosmetic ingredients into human skin due to the controlled drug release of BC (Pacheco et al., 2018; Oliveira et al., 2022). Figure 3 summarizes the different application areas of BC in cosmetics.

The usability of BC in cosmetics has been mainly investigated as a carrier of active ingredients or as a structuring agent of cosmetic formulations. With the ongoing sustainable new product development in the cosmetic industry and the growth prospects of the bio-based product market, BC is taking on a much more prominent role in this field. BC, a versatile polysaccharide produced by non-pathogenic acetic acid bacteria, is of interest in the skin care industry as a possible alternative to synthetic polymers (polyacrylamides, nylon, and polyethylene) frequently used in the cosmetics sector (Almeida et al., 2021).

BC serves as a polymeric material in the cosmetic industry that effectively binds active ingredients to the skin (Almeida et al., 2021). Essential oils, plant extracts, and many other active biochemicals can be more easily bound by hydrogen bonding in the BC polymer (Rizzi et al., 2021). This property is also important for drug delivery applications of BC (de Amorim et al., 2020).

Over the years, BC's suitability for use in cosmetics has been studied and proven, especially when used as a backing material for sheet face masks that apply active ingredients to the skin. But BC has also been utilized as a structuring agent in formulas for personal care products or natural peeling cosmetics (Ullah et al., 2016).

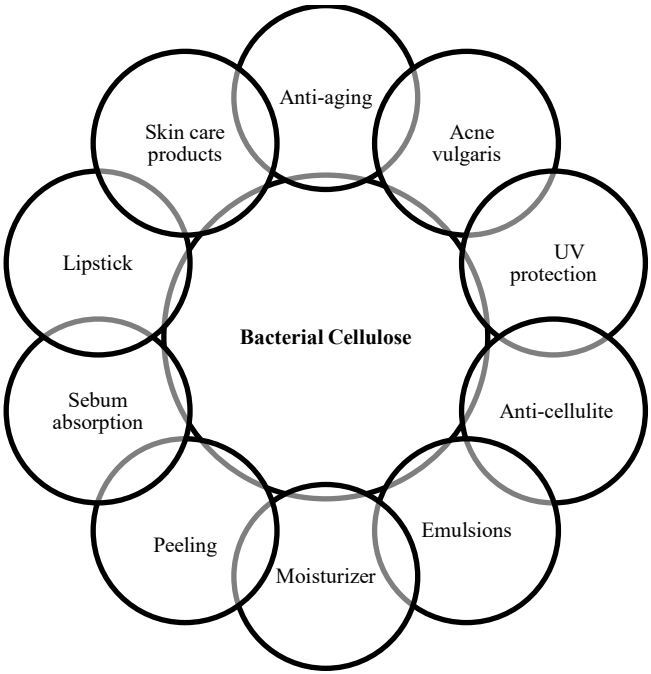

Figure 3. Cosmetic applications of bacterial cellulose

Acne vulgaris, a prevalent and bothersome facial skin issue, impacts around 80% of individuals, and it significantly affects social interactions, particularly during teenage years (Alba et al., 2017). The pathogenesis of acne is primarily caused by abnormalities in the colonization of normal flora, such as *Propionibacterium acnes*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (Phimnuan et al., 2019). Phimnuan et al. (2019) formulated a biocellulose film (*Acetobacter xylinum*) containing *Punica granatum* (pomegranate) peel extract to be used as an anti-acne product. This product was reported to be a natural polymer for acne treatment. In a study, the produced BC film produced by *Gluconacetobacter hansenii* was loaded with natural propolis extract for use as a skin care product to treat and heal acne-prone skin. The polymer blend formed offers beneficial properties for use as a BioMask in the cosmeceutical industry (Amorim et al., 2020). Antimicrobial amino acid

derivatives impregnated with BC (*K. xylinus*) have also been produced as anti-acne pharmaceutical preparations effective against *Cutibacterium acnes* (*P. acnes*) and *S. aureus* (Golonka et al., 2021).

Anti-aging skin care cosmetics frequently contain natural bioactive compounds (Cavinato et al., 2017). Particularly abundant in bioactive compounds are plant extracts (e.g., polyphenols, terpenoids, vitamins, and other substances) that may have a variety of skin-related effects (Ribeiro et al., 2015). Thus, in light of the current cosmetics trend toward natural products, plant extracts seem to be one of the most promising ingredients for skin care product development. Pacheco et al. (2018) prepared a mixture of BC, oat extract, rosemary extract, calendula extract, and hydroviton as a moisture enhancer in the herbal essence mask (VEM) formulation. A mixture of propolis/polypropylene glycol and BC was also prepared as a moisture reducer in the propolis extract formulation. It has been shown that BC nanofiber membranes can be used in skin care applications. Nascimento et al. (2022) obtained BC supplemented with grape skin extract, which has antioxidant and moisturizing properties, in standard HS medium with *Komagataeibacter hansenii* bacteria and in the medium containing grape peel extract in different compositions. In another study, BC with antioxidant and high water-retention properties added with propolis was produced (Amorim et al., 2022). Almeida et al. (2022) obtained an anti-aging facial mask composed of BC membranes, glycerol (as a plasticizer and moisturizer), and *Eucalyptus globulus* leaf extract. In 2 patent studies, BCs with a high moisturizing effect were obtained by adding bamboo extract (Ho et al., 2015) and *Moringa oleifera* leaf powder (Yuefeng et al., 2019) to the BC production medium. BC produced by *K. xylinus* was loaded with 5% and 10% ethanolic extract of *Epilobium angustifolium* and the resulting BC membrane was developed as a product for topical antioxidant delivery to the skin (Nowak et al., 2021). In a different investigation, a product for dermocosmetic applications was developed based on hyaluronic acid (HA) soluble with BC produced by *G. sacchari*. HA was used as an active biomacromolecule with

moisturizing and regenerating properties and a volumizing effect, while BC was used as a support for the incorporation of an additional bioactive molecule. This product has shown its potential for skin applications in cosmetics, taking advantage of the known properties of HA and the capacity of BC to control the release of bioactive molecules (Fonseca et al., 2021).

UV exposure can harm the skin's lipids, proteins, and cellular DNA through photo-oxidative damage (Fernandes et al., 2021). As a result, exposure to UV light can cause erythema, skin aging, and skin cancer (Walia et al., 2019). BC membranes containing plant phenolic compounds could be a biotechnological substitute for UV-induced skin damage prevention and treatment (Fernandes et al., 2021). BC was grown in static culture in the presence of silk nonwoven fabric (SNF) and impregnated with silk sericin (SS) via simple solution adsorption to impart strong UV protection properties to this material. This novel biocomposite membrane is promising for health and skin care applications such as wound dressings and beauty products (Wang et al., 2023).

Vitamins (A, B3, B8, C, D, E, K, and Coenzyme Q10) represent an important component of many cosmetic products, as they regulate numerous biological functions that affect skin health (Dattola et al., 2020). Vitamin B-based ionic liquids were incorporated into BC membranes, anticipating their use in skin care applications (Chantereau et al., 2020).

Caffeine was loaded into BC produced by *Gluconacetobacter sacchari*, and the obtained cellulose membranes were reported to have potential use in cellulite alleviation (Silva et al., 2014).

Cosmetic emulsions are composed of many surfactant systems (Xiao et al., 2021). Surfactants have been shown to cause adverse reactions such as skin irritation, hemolysis, and cytotoxicity (Varvaresou & Iakovou, 2015). Nowadays, cosmetic preparations use herbal natural products to avoid using chemicals (e.g., parabens)

that may have undesirable side effects such as skin allergies (El-Gendi et al., 2022). BC has been applied in cosmetics as a non-allergic biopolymer widely used to stabilize oil-water emulsions without the need for the addition of other skin-irritating surfactants. In a study, cosmetic creams consisting of oil-water emulsions were successfully prepared using BC and carboxymethyl cellulose instead of commonly used chemical surfactants (Martins et al., 2021). This mixture is clearly advantageous compared to chemical surfactants that cause some skin irritation problems. A patent describes a composition in the form of an oil-in-water emulsion containing an oily phase dispersed in an aqueous phase as being free of surfactants and containing cellulose fibrils with a length greater than 1 micron and a length/diameter ratio greater than 30. The invention also relates to the use of the composition, in particular for the care, treatment, make-up or cleansing of the skin, lips, eyelashes, and/or hair and for the care of sensitive or dry skin (Tournilhac & Lorant, 2003).

In another patent study, a cosmetic composition containing BC fragments was obtained. It was stated that the addition of BC film fragments to a cosmetic mixture not only improves the transdermal delivery of active ingredients contained in the cosmetic composition but also provides skin moisturizing, skin exfoliation, and sebum absorption functions. Various applications of the BC thus obtained were stated. In the same patent study, it is stated that BC film can be used in nail, skin and lip care, long-lasting perfume design, weight loss, eye bags or patches. Since BC has a high water content and good gas permeability, it is suitable as a substrate to carry cosmetic active ingredients and provide enhanced transdermal absorption (Lin et al., 2015).

In a patent study, a BC facial mask with anti-radiation effect was obtained by using herbal extracts. The prepared facial mask is good in water binding capacity and air permeability, and it is stated that it has good anti-radiation effect (Patent No CN101792735B). Lipstick is produced by incorporating natural water or oil soluble

pigments into biological/bacterial cellulose gel particles (Patent No CN112472611A).

There are many uses for contact lenses besides treating eye conditions, such as aesthetic or decorative ones (Steinemann et al., 2005). Decorative hydrogel lenses may not have high enough oxygen permeability to prevent hypoxic stress with daily wear or, more importantly, with continuous wear. In a patent study, a microbial cellulose material suitable for use as a lens has been developed with a convex surface that covers the cornea and is shaped according to the shape of the eye (Levinson & Glonek, 2010).

BC is an excellent alternative for a variety of cosmetic uses, such as skin treatments and emulsion stabilization, due to its excellent biocompatibility and biodegradability. The capacity of BC to serve as a carrier for active compounds improves their potency and distribution, which promises well for skincare products (Oliveira et al., 2022; Lima et al., 2024).

Conclusion

In this section, studies on the use of BC in the cosmetic field have been evaluated, and it has been shown that it is a very promising area for researchers. Studies have shown that BC is an ideal cosmetic biomaterial as a skin care product with its moisturizing properties, oiliness control, anti-aging, controlled substance release, and biocompatibility. Its biggest advantage is that it can be used safely because it does not cause any harm to the consumer. In the cosmetics industry, BC can be used for makeup, facial masks, simple peeling cosmetics, and personal care products. The use of BC is still limited in spite of its exceptional performance and unique advantages. However, it is expected that both research and commercial interest in BC for green cosmetics will expand in the upcoming years because to the expansion of the personal care industry and the growing demand for green products.

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CHAPTER XI

The Matrix Metalloproteinases in Humans

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1. Introduction

Malignant tumors pose a serious threat to worldwide human health. Early diagnosis of tumors is the main way to completely cure tumors, but it is also imperative to evaluate the complex process of tumor formation and development, tumor suppressor genes, oncogenes and the microenvironment tumor in tissue. The continuity of cellular development in the tumoroid structure requires appropriate physiological conditions for this development to spread to other tissues. The interactions that occur between malignant tumors and stromal cells such as endothelial cells, inflammatory cells and fibroblasts in the tissues can provide appropriate

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physiological conditions for the development and metastasis of tumor cells. Therefore, it can be stated that mesenchymal epithelial interactions play a vital role in the pathophysiology of many types of cancer. In addition to the interstitial cells in the structure of the tumor in tissues, the extracellular matrix (ECM) part also seems to be important in terms of the development of the tumoroid structure and its spread to other areas (Zong et al., 2024). It is known that the main components of the ECM are protein structures consisting of polypeptides such as collagen, elastin, fibronectin, laminin and fibrin. Matrix metalloproteinases (MMPs), which are proteases that contribute to the differentiation of the ECM and the spread of cancer, function in morphogenesis, bone tissue regeneration and wound healing under normal physiological conditions, but otherwise function in the degradation of various ECM proteins in cancer stages such as irregular regeneration of the extracellular matrix, invasion or metastasis (Fig 1) (Chen K, 2023; Zong et al., 2024). While it is reported that there are 24 types of 28 MMPs for humans, MMPs can be briefly divided into collagenases, gelatinases, stromelysins, matrilysins, membrane type (MT) MMPs and others. MMP enzymes share common features in terms of structure, consisting of a zinc ion catalytic domain, hemopexin and propeptide domain. However, the hemopexin domain is not found in matrilysins and MMP-23 (Chang, 2023).

ECM is an important function in that it provides mechanical support to cells in tissues, as well as containing many growth factors (vascular endothelial or transforming growth factor, etc.) and acting as a reservoir. In the healthy body, MMP activity is low when there is no serious physiological activity, but increases during inflammation or cellular healing when physiological activities are intense. In addition to cellular adhesion, MMPs also support the secretion of extracellular matrix molecules that enable microenvironmental signals to reach cells and enable them to respond to stimuli (Fig 1). The biological activities of MMPs are inhibited by tissue biosynthetic inhibitors (Wang et al., 2024).

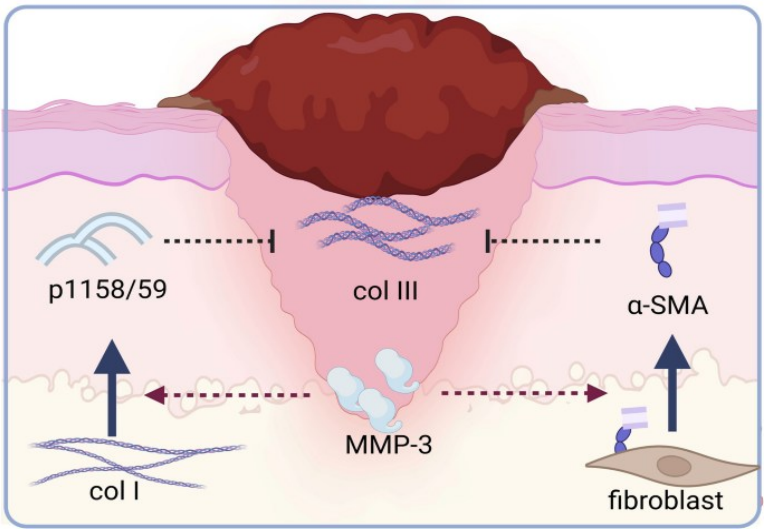

Figure 1. *MMP in extracellular matrix (ECM) remodeling and tissue regeneration. MMP-2 has functions in the formation of new vessels. During wound healing, increased MMP-3 activity has been found to promote skin repair. Increased MMP-3 activity has been reported to alleviate scar formation by reducing the level of collagen III and alpha-smooth muscle actin (α-SMA). The solid line represents the actual physical origin or composition of the molecule or matrilinear; the dashed line represents the stimulatory (red) or inhibitory (blue) function of MMPs (Chen et al., 2023).*

MMPs may play an important role in the release of growth factors and the degradation of proteins in the ECM structure that expose areas that can stimulate growth factor (GF) receptors. GFs also help determine the stability of the ECM structure by stimulating the expression of MMPs that alter the cells or ECM structure to increase the production of many molecules found in the ECM structure. From this perspective, GFs have a two-way effect on the ECM. During the process of wound transformation into their natural structures, keratinocytes at the wound edge separate from the basement membrane and move along the wound bed, initiating MMP production. The level of MMP enzymes is determined by the

interactions of the ECM, cells and molecules. Accordingly, it can be said that the ECM affects the regulation of cellular gene expression. Although the synthesis of proteolytic enzymes at appropriate levels requires great sensitivity for the normal healing of the wound, chronically increased levels of MMPs, whose levels increase during wound healing, can cause damage to the matrix structure (Schultz and Wysocki, 2009). When considered in this way, the modulatory effect of MMPs on the homeostatic balance concerning intercellular and ECM has a remarkable feature (Rashid and Bardaweel, 2023). The presence of MMPs is related to the pathophysiology and immune potential of many diseases such as invasion or metastasis of tumoral structure during cancer or cancerization, arthritis, osteogenesis imperfecta, periodontal, central nervous system or cardiovascular health problems. MMP enzymes synthesized and secreted by the cells of the immune system function in both innate and adaptive immunity. When the living organism is at rest, immune system cells synthesize low levels of MMPs. With the increased synthesis level of cytokine and chemokine proteins in the immune response against inflammation, MMPs are secreted and activated by immune system cells. In the formation of the response given in the immune system, the chemotactic shift of neutrophils from white blood cells, their passage to infection sites through blood vessels and tissues, and extravasation is an important feature. MMPs contribute to this process of chemotactic agents (He et al., 2023).

1.1. Structure of MMPs

MMPs structurally consist mainly of the following from N-terminus to C-terminus (Fig 2) (Sagi and Gaffney, 2015): (1) a signal peptide with hydrophobic properties; (2) an N-terminal propeptide that is cleaved by exogenous enzyme catalysis to activate the zymogen form of the MMP; (3) a catalytic (CAT) domain with a zinc ion binding site that hydrolyzes and cleaves peptide bonds; 4) a linker domain; and (5) a C-terminal hemopexin-like domain that recognizes substrates. Furthermore, transmembrane type (MT) MMPs contain a transmembrane (TM) domain responsible for intracellular activity and a cytoplasmic tail region, while MT-MMPs

bind to the cell membrane through glycosylphosphatidylinositol (Sagi and Gaffney, 2015; Zhao et al. 2023).

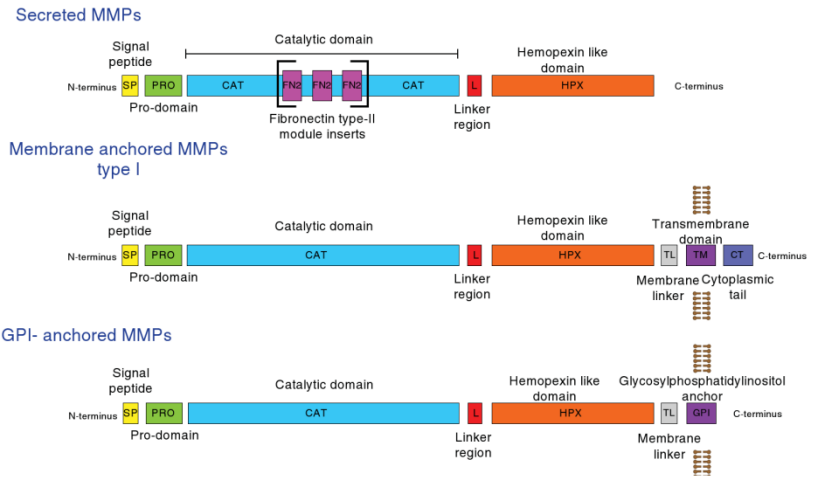

Figure 2. *General domain organization of MMPs*

1.2. Classification of MMPs

Twenty-eight MMPs have been identified in vertebrates, and 24 of them have been found in humans. Based on their sequence similarity, domain organization, and substrate specificity, MMP family members can be divided into (1) collagenases, (3) gelatinases, (3) stromelysins, (4) matrilysins, (5) transmembrane type I, (6), transmembrane type II, (7) glycosylphosphatidylinositol-anchored (GPI-anchored), and (8) other MMPs (Niland and Eble, 2020). Collagenases (MMP-1, MMP-8, MMP-13, and MMP-18) play a role in the degradation of essential components of bone tissue structure. Gelatinases (MMP-2 and -9) are involved in angiogenesis and neurogenesis. Stromelysins (MMP-3, MMP-10 and MMP-11) and matrilysins (MMP-7 and MMP-26) play a role in the digestion of structural parts of the ECM (Rashid et al., 2023).

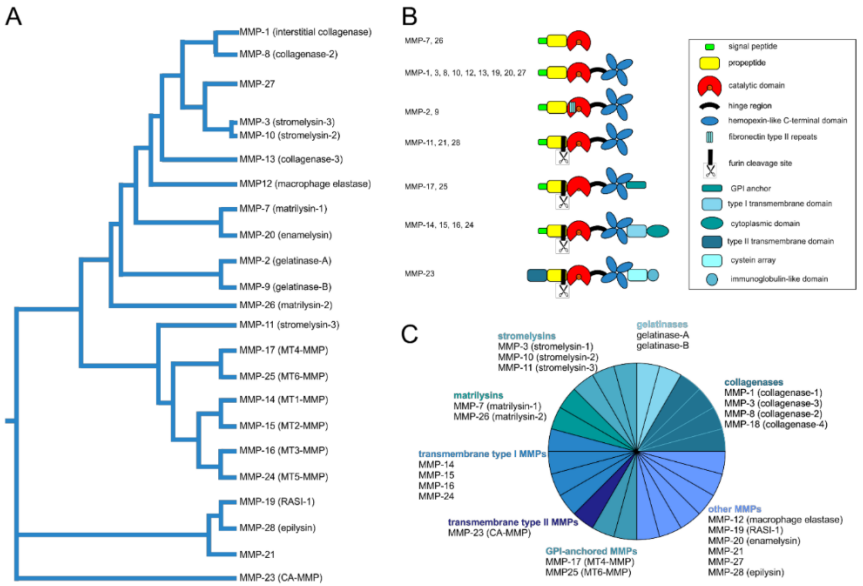

Figure 3. *Phylogenetic and functional relationships of human matrix metalloproteinase enzymes*

MMPs can also be ranked according to the order in which they were discovered and common features of their sequences. (A) According to the domain organization of MMPs, (B) and according to substrate specificity, (C) soluble collagenases, gelatinases, stromelysins, matrilysins, membrane-anchored transmembrane type I and type II and glycosylphosphatidylinositol (GPI)-anchored MMPs, and other MMPs. All MMPs except MMP-23 have a propeptide with an N-terminal signal sequence. To activate an MMP enzyme, this propeptide must be cleaved to form a zinc ion that is accessible by a cysteine key in its catalytic moiety and that also retains calcium ions (Fig 3) (Niland and Eble, 2020).

1.2.1. Collagenases (MMP- 1, -8, -13, -18)

Collagenase enzymes, including MMP-1 (mesenchymal collagenase), MMP-8 (neutrophil collagenase), MMP-13 and MMP-18, have an important effect on the molecular degradation of triple helical collagen structure and function and contribute to osteogenic

differentiation in bone tissue regeneration (Chung et al., 2004). MMP-1 enzyme is also known as collagenase-1 or interstitial collagenase enzyme molecule and is effective in eliminating the structure and function of collagen (I, II, III, VII, VIII, X), casein, entactin, laminin, pro-MMPn -1, -2, -9 and serpins. It has been reported that MMP-1 enzyme activity levels are very low under physiological conditions but increase in inflammation and autoimmune diseases with opposite conditions (Mittal et al., 2016). MMP-1s support epithelialization during the transformation of wounds into healthy normal cellular tissue by binding to type 1 collagen molecules in the dermis layer and indirectly activating cell membrane α5β1 receptors as a result of catalytic effects (Chen et al., 2023).

In addition, MMP-1 also contributes to human bone marrow mesenchymal stem osteogenic differentiation by using JNK and ERK metabolic pathways (Wu et al., 2020). MMP-13 enzyme is effective in the destruction of type II collagen structure and function and is therefore an essential MMP enzyme that functions in the deterioration and destruction of cartilage tissue structure. Moreover, MMP-13 is reported to be effective in the adaptation of osteogenic structure and function of human mesenchymal stem cells (hMSCs) to certain lineages during self-renewal and healing (Hu and Ecker, 2021; Chen et al., 2023).

1.2.2. Gelatinases (MMP- 2, -9)

MMP-2 and MMP-9 enzymes are collectively known as gelatinase enzymes and are also referred to as MMP-2 gelatinase-A and MMP-9 gelatinase-B. Although gelatinases are structurally similar to other proteinases in the MMP family, it has also been noted that the enzyme molecule has a different collagen molecule binding domain containing three fibronectin type II tandem repeats at the N-terminus of the catalytic domain, which is suitable for gelatin binding (Das et al., 2022). Gelatinase enzyme molecules are usually synthesized and secreted from vascular smooth muscle cells, leukocytes, and fibroblasts. While MMP-2 enzyme is mainly

synthesized and secreted by leukocytes, platelets, endothelial cells, chondrocytes, keratinocytes, osteoblasts, monocytes and dermal fibroblasts, MMP-9 is reported to be secreted by cell types such as granulocytes, fibroblasts, polymorphonuclear leukocytes, neutrophils, macrophages, T cells, osteoblasts, keratinocytes and dendritic cells of epithelial cells (Das et al., 2021).

By cleaving N-calmodulin, MMP-2 can loosen the connections between vascular smooth muscle cells and promote cell migration and proliferation. It is noted that overexpression of MMP-2 can promote angiogenesis by stimulating VSMC activity through the continuous secretion of cytokines and growth factors, which are immune molecules from the ECM (Seliktar et al., 2004). The protease activity of the MMP-2 enzyme also promotes the synthesis, secretion, and activation of ECM-associated angiogenic growth factors, including vascular endothelial growth factor (VEGF), tumor growth factor β (TGF-β), and basic fibroblast growth factor (bFGF), and metastatic stimulation is also provided (Rundhaug, 2005; Chen et al., 2023).

1.2.3. Stromelysins (MMP- 3, -10, -11)

MMP-3, MMP-10 and MMP-11 are included in the stromelysins group. Although stromelysins have the same domain arrangements as collagenases -1, -2 and -3, they do not show a degradative effect on interstitial collagen. MMP-3 and MMP-10 show similar common activities on the substrate, such as breaking down and rendering dysfunctional the proteinic structures on the extracellular matrix and cell surface. These enzymes activate other precursor MMP enzymes by cutting and destroying propeptides. The MMP-11 enzyme can degrade extracellular matrix components more effectively than other MMPs. MMP-11 is activated while inside the cell to perform its function and begins its activity by secreting it as an active enzyme. Stromelysins have the feature of angiogenesis-promoting enzymes in the regeneration of the extracellular matrix. MMP-3 enzyme has the ability to support the effectiveness of immunity and the increase in vascular permeability by enabling the

movement of neutrophil and monocyte cells from the blood vessels in the injury areas by proteolysis of basal lamina components and tight junction protein structures in the tissue at the initial stage of inflammation (Parks et al., 2004).

1.2.4. Matrilysins (MMP-7, -26)

The simplest and smallest enzymes of MMPs are matrilysin-1 (MMP-7) and matrilysin-2 (MMP-26). Compared to other MMPs, they reflect each other's structure in terms of structural characteristics, having only pre-peptide and catalytic effects in their structures, but lacking the hemopexin domain and hinge region. Matrilysin molecules show broad substrate specificity on the proteinic structures of the extracellular matrix, basement membrane and cell surface components (Piskór et al., 2020; Chen et al., 2023). While the MMP-7 enzyme molecule is expressed in Xenopus embryonic macrophage cells from a cellular perspective, the most common substrates of these enzymes for the organism are fibronectin, casein, proteoglycans and type I, II, IV and V gelatins. MMP-7 enzymes play a role in the remodeling of developmental and reproductive tissues with high proliferative potential, such as the uterus, and in tissue remodeling after injury in normal tissues. The MMP-7 enzyme proteolyzes extracellular matrix components and involves cell surface molecules such as Fas-ligand, pro-TNF-α, syndecan-1 and E-cadherin in proteolytic reactions, resulting in the formation of new soluble structures. The MMP-7 enzyme may have dual functions, with the ability to induce apoptosis by releasing Fas-ligand on apoptosis and inhibiting apoptosis by producing heparin-binding epidermal growth factor. The MMP-7 enzyme may also have an important role in proteolytic catalysis reactions within cells in the intestine to process proscriptins into bactericidal forms (Verma and Hasch, 2007; Khalil, 2017; Pehlivan and Oyacı, 2022). It is noted that the MMP-26 enzyme, also known as matrilysin-2 or endometriase, has a gene locus on chromosome 11p15 and its mRNA can be expressed especially in the uterus and placental tissue. It is also reported that recombinant MMP-26 enzyme exhibits degradative activity in terms of proteolytic reaction against many

substrates including molecules such as β-casein, fibrinogen, collagen type IV, gelatin and fibronectin. It has also been stated that MMP-26 enzyme activates proMMP-9 enzyme molecule (gelatinase B) (Pehlivan and Oyacı 2022). It is also reported that MMP-26, also called endometritis, which activates pro-MMP-9 enzyme, was identified from fetal cDNA and its 261 amino acid sequence shows 51.8% homology to macrophage metalloelastase (Altemeier et al., 2012; Chen et al., 2023).

1.2.5. Membrane Type MMPs (MT-MMPs)

Membrane-type MMP enzymes (MT-MMPs) are represented by four kinds of transmembrane MMPs (MMP-14, -15, -16 and -24) and two glycosylphosphatidylinositol (GPI)-anchored MMPs (MMP-17 and -25). MT-MMPs are named because they can be membrane-bound and have proteolytic activities on the surface of cells. Among MT-MMP enzymes, MT1-MMP is known as a transmembrane protease enzyme with a short cytoplasmic tail that acts in the remodeling of the extracellular matrix. MT1-MMP degrades type I-III collagen, fibronectin, laminin-1, hyaluronan, cartilage proteoglycans, α2-macroglobulin and α1-protease inhibitor. MT1-MMP has the ability to bind to both matrix metalloproteinase and tissue inhibitor-2 (TIMP-2), activate MMP-2, and disrupt collagen networks. Thus, cancer cells can facilitate the passage of various cell types, including fibroblasts and endothelial cells, through these barriers from the extracellular matrix, thus promoting all cancer processes. MMP-2 enzyme also has the ability to degrade type IV collagen, an important component of BM. Therefore, activation of pro-MMP-2 by MT1-MMP is considered an indication that BM plays a critical role in cell invasion, regeneration, and angiogenesis (Kang et al., 2019; Chen et al., 2023).

1.2.6. Metalloelastase (MMP- 12)

The expression of the MMP-12 enzyme molecule was first discovered in the alveolar macrophages of smokers. MMP12 may contain an amino-terminal propeptide domain that can regulate the latent or potential effect of the enzyme, a catalytically effective zinc

and calcium binding domain, and a carboxy-terminal hemopexin equivalent domain that is effective in substrate selectivity. Similar to other MMP enzymes, MMP12 is associated with a sequence in the MMP-encoding gene cluster on chromosome 11q22.3. In addition to its proteolytic activity on molecules in the relevant tissues of the living organism, it has also been reported that the MMP12 enzyme has the ability or property to activate pro-MMP-1 and pro-MMP-9 enzymes (Matsumoto et al., 1998). This enzyme can also disrupt and eliminate the function of extracellular matrix components with many different structures and properties such as fibronectin, laminin, collagen type IV, heparan sulfate and chondroitin sulfate proteoglycan. It has been reported that the MMP12 enzyme has a wide range of effects in living organisms, including regulating reproduction, embryonic development and tissue remodeling, playing a tissue integrity or antiinvasive role by damaging the natural structure of the basement membrane, enabling macrophage cells to enter the injured tissue for defense and protection during inflammation during the process in which immunity is stimulated, a significant damaging effect in the case of pulmonary emphysema characterized by the formation of large-area air sacs, antitumor, anti-inflammatory, antibacterial and antiviral effects, and wound healing and repair functions (Lin et al., 2023).

2. Expression of MMPs

MMP expression is precisely driven by the signaling of NF-κB, MAPK and JAK/STAT pathways, cell-cell interactions and cell-matrix, growth factors, cytokines, retinoic acid, glucocorticoids, eicosanoids and interleukins. Some of the MMP enzyme promoters are co-regulated due to common motifs in regulation and structural effects (Chatterjee et al., 2018, Yan and Boyd 2007; Niland et al., 2022). MMP enzymes can be classified among themselves in terms of their substrate specificity, but it is also possible to examine them in three groups in terms of the way gene expression is regulated. The largest group among these enzymes is represented by MMP-1, -3, -7, -9, -12, -13, -19 and -26, all of which contain a TATA box and an AP1. The second MMP group consists of MMP-8, -11 and -21

enzymes, which have a TATA box but no AP1 (Niland et al., 2022). Another group includes MMP-2, -14 and -28 enzymes, but lacks both the TATA box and AP1. They are constantly expressed constitutively, but they are overexpressed when experiencing certain health problems. The EGF receptor, which is constantly stimulated and ready to be stimulated in many types of cancer, may have the ability to simultaneously activate many MMP enzyme genes and other genes. In the system involving mesenchymal and monocytic cells, inflammatory signals such as TNF-α cytokines, IL-1β, microbial lipopolysaccharides and oncostatins stand out as the most powerful transcription activators of MMP-1, -3, -9, -13 and -14 enzymes (Vincenti and Brinckerhof 2007; Niland et al., 2022).

MMP enzyme synthesis can be indirectly regulated by microRNAs and trans-acting RNA binding proteins after transcription, as they provide mRNA stability. An example of this is that MMP-14 enzyme expression can be reduced by miR-181a-5p and miR-7, thus inhibiting cancer cell migration and angiogenesis in cancerous tissue or organism. Long non-coding RNAs (LncRNA) also have the ability to reduce the expression of MMP-2, -9 and -14 enzymes by interacting with miR-142-5p, as in the effect of bladder cancer-associated transcript-1 (BLACAT1). It has also been reported that the expression of MMP enzymes can be controlled by circular RNAs that bind to miR-518c-5p, reducing or eliminating the inhibitory effect of miRNA, which increases MMP-2 enzyme expression (Niland et al., 2021).

The regulation of the molecules in terms of ratio after translation processes may require phosphorylation, partial proteolysis, glycosylation, activation with furin, and interactions with intracellular and extracellular protein and lipid molecules (Niland and Eble, 2020). MMP enzymes may also have the ability to directly or indirectly affect each other's activities or expressions. The ability to proteolyze and cut the catalytic part of the MMP-14 enzyme in the pericellular area or to reversibly or irreversibly inactivate the MMP-11 enzyme using the TIMP-mediated inhibition

pathway can be given as a good example of this (Buache et al., 2014; Niland et al., 2021).

Among the MMP enzymes, MMP-14 has a central role (Fig 4) (Niland and Eble, 2020; Niland et al., 2022). MMP-14 enzyme expression is tightly regulated from the transcription stage of mRNA synthesis to all processes involving post-translational modifications. MMP-14 gene expression is regulated by histone modification, chromatin remodeling, and DNA methylation-sensitive transcription factors such as SP1. The MMP-14 promoter has a binding site for the repressive transcription factor PROX1, which can significantly affect the invasion of cancer cells by reducing MMP-14 enzyme expression. Collagen interactions and mechanical forces in the tumor microenvironment, via a pathway involving α2β1 integrin, cause an increase in MMP-14 enzyme expression via the transcription factor early growth response protein-1. The MMP-14 enzyme molecule is activated by a pathway based on the removal of the N-terminal propeptide by proprotein convertase furin catalysis. This is mediated by the use of Golgi recombinant stacking protein-55, which acts as an adaptor when passing through the trans-Golgi compartment, so that the MMP-14 enzyme can reach the surface of the cells as an activated enzyme molecule. MMP-14 enzyme packages occur in intracellular vesicles that bind to microtubules via motor proteins (Niland and Eble, 2020; Niland et al., 2022).

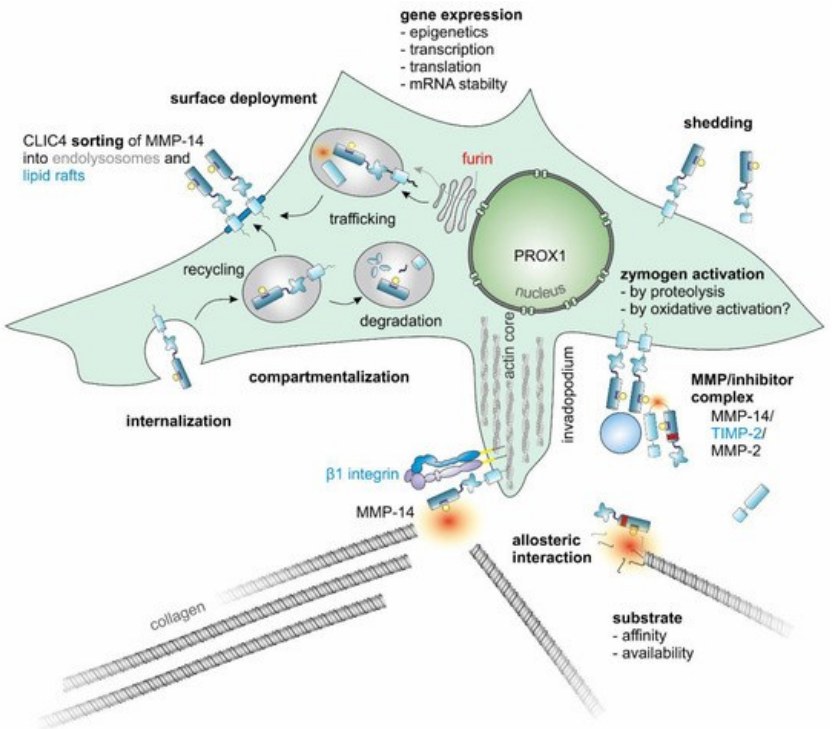

Figure 4. *The enzymatic activity of MMP-14 with complex regulation*

3. Activation and Inhibition of MMPs

Various intracellular protein molecules such as nuclear lamins, cytoskeletal proteins, transcription factors, chaperones, apoptosis and translation regulators are recorded as substrates of MMP enzymes. In order to prevent these molecules from being exposed to accidental proteolytic effects, cellular interactions and various factors such as cytokines, glucocorticoids and interleukins are sensitively active. The expression of MMP enzyme molecules can be induced through metabolic pathways involving JAK, NFκB and MAPK (Niland and Eble, 2020; Niland et al., 2022).

There are various metabolic effects or pathways that cause enzymes to work and stop while the living organism continues its

normal life. The importance of each of these effects or pathways may be at different levels as a result of impaired health conditions. The proteolysis process of MMP enzymes can be said to be regulated in four steps: (1) regulation of MMP gene expression using epigenetic, which includes chemical changes that occur in the genes of the organism during life and affect gene expression, and transcriptional control mechanisms, which is the process of copying the DNA sequence of a gene into an RNA molecule, (2) compartmentalization of MT-MMP enzymes in vesicles and membrane microdomains, which are spherical vesicles surrounded by a membrane, (3) production and activation of zymogen enzymes, which are inactive enzyme precursors when necessary, and (4) termination or inhibition of proteolytic processes (Löffek et al., 2011; Niland et al., 2022).

The degenerative potential of MMP enzymes for proteins, namely their proteolytic effects, can be well controlled at the transcription stage by activating zymogens and endogenous tissue metalloproteinase inhibitors (TIMPs) (Bode et al., 1999). TIMPs, which have small sizes, bind tightly to the CAT domain of MMP enzymes, and this binding can prevent substrates from being modified or degraded. Each TIMP in the protein family of four homologous TIMPs (TIMP1-4) specific for MMPs can inhibit various MMP enzymes with different inhibitory effects (Zhao P, 2023). The N-terminal domain in the TIMP molecule performs an inactivation function by chelating the catalytic zinc ion located in the active center of the MMP enzyme. Unlike this method, TIMPs can also activate the hemopexin and C-terminal domains of MMP enzymes by interacting with them (Niland et al., 2022). Collagen degradation is regulated by the catalytic effects of MMP enzymes, and MMPs are in turn regulated by TIMPs and MT1-MMP tissue inhibitors. The secretion of MMP enzymes by lymphocytes and granulocyte cells is regulated by transcription factors and epigenetic factors involved in the transfer of genetic information from DNA to RNA. Collagenases are secreted as zymogens to protect the site where they are synthesized from damage and are made functional through the stromelysin molecule (Fig 5) (Singh et al., 2023).

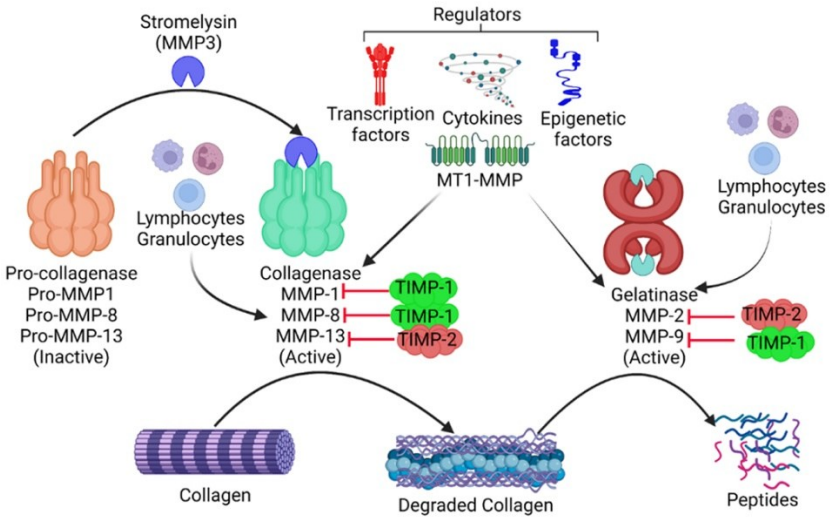

Figure 5. *Regulation of collagen proteolysis*

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CHAPTER XII

The Role Of Bıoınformatıcs In Advancıng Plant And Anımal Bıotechnology

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Introduction

Bioinformatics is defined as a multidisciplinary field that basically consists of biology, mathematics and statistical computer science, and enables the storage and processing of large amounts of complex data (Singh, Singh, Chand & Kushwaha, 2011; Khalid et al., 2021). However, technological developments in recent years have added a different dimension to the meaning and function of bioinformatics, especially in agriculture. Let's just imagine for a few minutes that we compare the genes of two plant or animal species without computer science; store and analyze the data, produce reliable statistical results, and predict potential needs for new

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experiments... How difficult it is to even imagine. The time required to complete this entire process, and the reliability of the results obtained are open to discussion... Especially in molecular genetic studies conducted at the population level in agriculture, it feels almost impossible to analyze thousands of genes/gene products from hundreds of samples and produce meaningful and reliable results (Xue, Zhao, Liang, Hou & Wang, 2008). With this view, bioinformatics has gained a meaning that strengthens and advances agricultural biotechnology. That is, as computer science has developed, the type and amount of biological data obtained has also changed over time.

Processing, managing, analyzing, obtaining meaningful and reliable results and visualizing the big data obtained from genomic technologies (also called 'omics') that have made great progress in recent years reveal the power of bioinformatics (Xue, Zhao, Liang, Hou & Wang, 2008). However, bioinformatics is not limited to these, for example, it can successfully realize high-level identification of target molecules from complex mixtures or high-accuracy identification and quantification of target genes/products involved in cellular regulatory processes, even from a single cell (Gomez-Casati, 2018).

Of course, as with many subdisciplines of molecular genetics, next-generation sequencing (NGS) has had a revolutionary impact on plant and animal biotechnology. NGS technology reads the fragments of millions of DNA/RNA by performing multiple reactions in parallel. Using this technology, the time and cost required for DNA/RNA sequencing has been significantly reduced and the analysis of the genomics, metagenomics, epigenomics and transcriptomics of many species has been performed thanks to NGS bioinformatics tools (Ohashi, Hasegawa, Wakimoto & Miyamoto-Sato, 2015; Pereira, Oliveira & Sousa, 2020). Thus, the collaboration of NGS and bioinformatics has made it possible not only to determine the quantitative characteristics of various genes but also to reveal the agriculturally important properties of genes (Li, Lin, An, Wang & Huang, 2018, Khalid et al., 2021).

One of the most frequently used methods of gene expression profile research is microarray technology. It basically allows the determination of the abundance for thousands of genes or transcripts as well as SNP genotyping and identification of transcription factor binding sites using the ChIP-chip method (Jaksik, Iwanaszko, Rzeszowska-Wolny & Kimmel, 2015). Both technologies, RNA-seq and microarray, are analyses that aim to identify genes for a specific trait (such as disease resistance or yield) and to describe biological pathways, and various bioinformatics tools are needed for the analysis, correct interpretation, and visualization of the data (Loewe & Nelson, 2011; Gomez-Casati et al., 2018).

With the advancement of genome-wide sequencing technology in recent years, genome-wide association studies (GWAS) have been developed for populations using dense genomic markers (He & Gai., 2023). The GWAS has accelerated quantitative trait locus (QTL) mapping and gene discovery in plants and livestock. Thanks to GWAS, the identification of genes underlying QTLs associated with economic traits in plants and livestock has been accelerated recently. This genetic approach has improved the resolution of QTL positions and helped to identify functional causative variations in genes by examining genotype-phenotype relationships (Le Nguyen, Grondin, Courtois & Gantet, 2019).

Significant progress in bioinformatics and molecular biology has led to transformative advancements in both plant biotechnology and animal biotechnology. In animal biotechnology, research focuses on areas such as nutrigenomics, animal health, breeding, and population genetics. In plant biotechnology, efforts are concentrated on pest control, genetic modification through gene-editing techniques, development of plant-derived medicines for healthcare, plant breeding, and crop production. Across all these domains, bioinformatics serves as an essential tool for statistical data analysis, results visualization, data storage in specialized databases, and the development of analytical workflows. This chapter highlights the applications of bioinformatics in both plant and animal biotechnology and its critical contributions to these fields.

Bioinformatics Approaches in Animal Biotechnology

Historically, animal breeding was primarily based on observable phenotypic traits and reproductive capacity. However, as the field of genetics has advanced, breeding methodologies have evolved. In particular, developments in quantitative genetics have enabled the integration of complex pedigrees into breeding programs. The combination of theoretical advancements and experimental successes has culminated in the creation of modern, genetically improved breeds (Singh, Gautam, Rao, Tandon & Kaur, 2018).

With advances in technology and molecular genetics, researchers have generated extensive genetic information that can address key challenges such as disease resistance and productivity **(**Cai, Duzsa, Guldbrandtsen, Lund & Sahana, 2020). Managing, analyzing, and interpreting this large, complex data—and converting it into actionable biological insights—requires robust bioinformatics tools and expertise. Consequently, the demand for databases that facilitate the efficient storage, access, and utilization of genetic information has grown (Adebayo et al., 2024).

Genetic Diversity Analysis

The advent of next-generation sequencing (NGS) has enabled the complete sequencing of DNA for numerous organisms. This advancement has facilitated the study of genetic diversity, particularly in farm animals, by analyzing changes in gene frequencies across populations and geographic regions. Quantitative geneticists incorporate these data into their analyses to study population structure, within- and between-population diversity, inbreeding coefficients, parentage relationships, linkage disequilibrium, and historical bottlenecks (Singh, Gautam, Rao, Tandon & Kaur, 2018).

A variety of genetic markers are employed in these analyses, including: Major histocompatibility complex loci (MHC), Restriction fragment length polymorphisms (RFLPs), Allozyme

loci, Microsatellites, Amplified fragment length polymorphisms (AFLPs), Mitochondrial DNA (mtDNA). Among these, microsatellite markers are widely used due to their species-specific conserved regions and unique properties. Recognized as priority molecular tools for the Measurement of Domestic Animal Diversity (MoDAD**)** by the FAO, microsatellites continue to be a cornerstone of genetic analyses (Zhu et al., 2000).

Several bioinformatics tools and software packages are used for genetic diversity analysis. These include: MEGA6 (Tamura, Stecher, Peterson, Filipski & Kumar, 2013), POPGENE (Pfeifer, Wittelsbürger, Ramos-Onsins & Lercher, 2014), PopGen32 (Yeh, Yang, Boyle, Ye & Xiyan, 2000), GenAlEx (Peakall & Smouse, 2006), Arlequin (Excoffier & Lischer, 2010), Structure (Pritchard, Wen & Falush, 2002), Phylip (Felsenstein, 1993), GENPOP (Rousset, 2008), DnaSP (Librado & Rozas, 2009) and Splitstree (Huson & Bryant, 2006).

In addition to these tools, modern programming languages such as R and Python provide advanced capabilities for storing and analyzing large genetic datasets efficiently. The open-source nature of the R programming language makes it particularly attractive for researchers, offering extensive data manipulation and visualization functionalities (R Core Team, 2020).

R Packages for Genetic Analysis

R-based bioinformatics workflows enable a variety of genetic analyses, including:

- Population genetics: Using packages such as *pegas* (Paradis, 2010), *adegenet* (Jombart, 2008), and *ape* (Paradis, Claude & Strimmer, 2008).
- Sequence alignment: Achieved through *msa* (Bodenhofer, et al., 2015).
- Haplotype network analyses: Conducted using *haplotypes* (Aktas, 2015) and *pegas* (Paradis, 2010).

- Phylogenetic tree visualization: Enabled by *ggtree* (Yu, Smith, Zhu, Guan & Lam, 2017) and *ggplot2* (Wickham, 2011).

These tools and workflows provide researchers with the ability to analyze complex genetic data, perform population structure assessments, and construct detailed phylogenetic trees, significantly advancing the field of animal biotechnology.

Marker-Assisted Selection (MAS)

The development of advanced genetic analyses has made it possible to identify the genes or gene regions that control specific traits and to understand the genetic mechanisms underlying these traits. With the advent of deep sequencing and efficient SNP genotyping using DNA chips, genomic selection for single or multiple traits within populations has become achievable (Singh, Gautam, Rao, Tandon & Kaur, 2018). These selection technologies rely heavily on bioinformatics tools to process and interpret the vast amounts of data generated (Goddard & Hayes, 2007). In this process, obtaining DNA sequence data and identifying polymorphisms are essential for selecting and improving animals for particular traits. Moreover, elucidating the effects of physically mapped genes, especially those influencing economically significant traits or quantitative trait loci (QTLs), is crucial for advancing genetic selection methods (Singh, Gautam, Rao, Tandon & Kaur, 2018; Cai, Duzsa, Guldbrandtsen, Lund & Sahana, 2020).

Marker-assisted selection (MAS) uses genetic marker information to predict breeding values and facilitate genetic selection. The process of MAS primarily occurs in two stages. First, markers that are in linkage disequilibrium with mutations affecting traits of interest must be identified as significant through genome-wide association studies (GWAS). These markers, often associated with QTLs, provide the genetic information required for selection. In the second stage, the identified markers are incorporated into

predictive models to estimate breeding values, enabling targeted genetic improvement (Hayes & Goddard, 2010).

To support these efforts, comprehensive databases such as the Animal QTL Database (Animal QTLdb; https://www.animalgenome.org/cgi-bin/QTLdb/index) and the GWAS Atlas (https://atlas.ctglab.nl/) provide publicly accessible resources for genetic data. The Animal QTLdb, which serves as a repository for quantitative trait mapping data, candidate genes, and genome-wide association studies, currently holds QTL information for seven species. The majority of the data pertains to cattle, with 192,247 recorded QTLs, followed by pigs (55,688) and chickens (18,602). Similarly, the GWAS Atlas is a valuable resource for genome-wide genotype-phenotype associations across diverse species. All curated biological trait data are mapped to standardized bio-ontologies, including the Animal Trait Ontology for Livestock (ATOL; https://www.atol-ontology.com/en/atol-2/) and the Animal Phenotype and Trait Ontology (APTO), enhancing the usability and accessibility of genetic information for research purposes (Liu et al., 2023).

In recent years, the integration of genetic data, phenotypic data, and pedigree information has enabled genetic selection to occur much earlier in the breeding process. This advancement has led to significant reductions in the generation interval, improved selection accuracy, and lower overall costs associated with breeding programs (Bouchard & McGue, 2003; Moreno et al., 2003). Consequently, the MAS method has proven particularly effective for traits with low heritability, traits linked to sex, those that are expensive to measure, or traits controlled by a limited number of genes (Singh, Gautam, Rao, Tandon & Kaur, 2018). By combining these advantages, MAS has emerged as a highly efficient tool for enhancing economically important traits in animal breeding programs.

Immunogenomics

Immunogenomics explores the genetic basis of immune system responses and their role in disease resistance and pathogenesis, particularly in farm animals and poultry. A significant portion of the vertebrate genome is composed of immunity-related genes, which are essential for survival (de Bono & Trowsdale, 2003). The innate immune system, as the first line of defense against pathogen invasion, relies on genes that are strong candidates for determining disease resistance (Loving, Osorio, Murtaugh & Zuckermann, 2015). The identification and characterization of these candidate genes, achieved through the integration of immunogenomics and bioinformatics, offers valuable insights into disease resistance, which can subsequently be applied in breeding programs.

Immunogenomics, which increasingly incorporates bioinformatics, investigates the genetic basis of immune responses that are linked to disease resistance and pathogenesis, particularly in farm animals and poultry (Pal & Chakravarty, 2020). This field has also led to the creation of specialized databases to organize and store immunogenomic data. For instance, the Avian Immunome DB compiles information on avian immune genes sourced from Ensembl, UniProt, and the Bird 10,000 Genomes (B10K) Project (https://b10k.genomics.cn/index.html) (Zhang, 2015; Mueller et al., 2020). This database focuses on immune system-related genes in chickens (*Gallus gallus*), categorized under immune system processes.

Advancements in immunogenomics have significantly improved the ability to identify candidate biomarkers for disease resistance. These discoveries have also facilitated the use of genome editing technologies to enhance disease resistance traits. Recent breakthroughs in gene editing tools, such as CRISPR/Cas9, have provided new opportunities for improving disease resistance in animal breeding. Despite variations in reproductive physiology across species, genome editing has proven successful in enhancing

economically important traits (Islam et al., 2020; Adebayo et al., 2024). The integration of immunogenomics with bioinformatics represents a powerful approach for identifying candidate genes associated with disease resistance. By leveraging these technologies, researchers can address challenges in animal health and breeding, thereby improving resistance to diseases and contributing to the development of resilient livestock populations.

Omics

The advancements in bioinformatics and molecular genetics have led to the establishment of numerous transcriptomic, genomic, and metabolomic studies, along with the development of animal genetics-specific databases. These databases, often powered by next-generation sequencing (NGS) technologies, serve as essential repositories for genome-wide DNA, RNA, and protein data across various species. Prominent databases include GenBank (www.ncbi.nlm.nih.gov), EMBL (www.ebi.ac.uk/embl.html), and DDB (www.ddbj.nig.ac.jp) for nucleotide and protein sequences, ExInt (http://sege.ntu.edu.sg/wester/iekb/) for exon-intron structures of eukaryotic genes, and SWISS-PROT (www.expasy.ch/sprot) for curated protein sequences. Additionally, databases such as KEGG (www.genome.ad.jp/kegg) focus on metabolic pathways, while DbSNP (http://www.ncbi.nlm.nih.gov/SNP/) serves as a comprehensive repository for single nucleotide polymorphisms (SNPs). For transcription factors across 183 animal genomes, AnimalTFDB (https://guolab.wchscu.cn/AnimalTFDB4//#/) provides critical genome-wide data, whereas EDomics (http://edomics.qnlm.ac/) integrates evolutionary developmental biology insights into genomics and transcriptomics information. Specialized databases for specific species have also been created, such as Sheep QTLdb (http://www.animalgenome.org/cgi-bin/QTLdb/OA/index) for sheep quantitative trait loci (QTL) data and the Chicken Genome Database (http://www.ncbi.nlm.nih.gov/mapview/map_search.cgi?taxid=903) for chicken genome assembly.

In addition to genomics databases, significant efforts have been made to create resources for tracking, documenting, and preserving genetic resources in animal genetics and husbandry. These databases provide detailed information on genetic resources, breed diversity, and the current status of various breeds worldwide. Key international databases include the Animal Genetic Resources - Information System (AGRI-IS; https://agris.fao.org/), the Domestic Animal Diversity Information System (DAD-IS; https://www.fao.org/dad-is/en/), and the Food and Agriculture Organization (FAO; http://www.fao.org/). Alongside these global initiatives, regional and national databases have also been established. Examples include the Animal Genetic Resources of Canada, the Centre for Genetic Resources of the Netherlands (CGN; https://agriculture.canada.ca/en/science/collections/animal-genetic-resources-canada), and the European Farm Animal Biodiversity Information System (EFABIS; https://www.fao.org/dad-is/regional-national-nodes/efabis/en/). Similar efforts have been undertaken in other regions, such as the Indian Council of Agricultural Research-National Bureau of Animal Genetic Resources (ICAR-NBAGR; https://nbagr.icar.gov.in/en/home/), the European Regional Focal Point for Animal Genetic Resources (EUGENA; https://www.eugena-erfp.net/en/), and the African Union Interafrican Bureau for Animal Resources (AU-IBAR; https://www.au-ibar.org/). The National Animal Genetic Resources Centre and Data Bank of Eastern and Central Africa (NAGRC&DB; https://nagrc.go.ug/) also plays a vital role in preserving genetic diversity and ensuring sustainable use of animal resources.

Animal Ethics and Welfare (In-Silico Models)

With advancements in biological sciences, the growth of medical and veterinary research, and the expansion of the pharmaceutical industry, there has been a significant increase in the use of experimental animals (Russell & Burch, 1959, reprinted 1992). To address ethical concerns and improve the welfare of laboratory animals, the 3Rs framework—Replacement, Reduction,

and Refinement—was developed. First described by Russell and Burch (1959), the 3Rs principles aim to achieve more humane research practices by minimizing animal stress and reducing the number of animals used in experiments. Over time, additional approaches were introduced, such as the Read-across method outlined in Annex XI of the REACH regulation (Registration, Evaluation, Authorisation, and Restriction of Chemicals, Regulation EC 1907/2006). This method estimates endpoint information for a target substance using data from the same endpoint of a comparable source substance (Rovida et al., 2020). To further advance these efforts, the European Partnership for Alternative Approaches to Animal Experiments (EPAA) organized the Partners Forum on Toxicokinetics and Read-Across. This initiative focused on developing in vitro toxicokinetic methods and physiologically based kinetic models, fostering synergies to enhance the use of toxicokinetic data and strengthen read-across approaches (Laroche et al., 2018). Collectively, these advancements have underscored the need for generating toxicokinetic data through in vitro and in silico tools.

In silico models enable the prediction of chemical properties and interactions based on the structure of the targeted chemical. By encoding the structural features, potential interactions, and effects of a chemical into its molecular framework, these models facilitate the development of structure-activity relationship (Q(SAR)) or structure-property relationship (Q(SPR)) models for a wide range of substances. This approach is grounded in the principle that chemicals with similar structures exhibit similar properties (Madden, Enoch, Paini & Cronin, 2020). However, challenges arise when evaluating differences in properties rather than similarities, adding complexity to these analyses (Cruz-Monteagudo et al., 2014).

The application of in silico models spans various areas, including the assessment of pollutant toxicity, evaluation of agricultural chemical effects on environmental species, and optimization of drug candidates. To support these applications,

numerous freely accessible databases store toxicological data, structural information, physicochemical properties, and safety assessments of chemical products. Examples include AMBIT (https://cefic-lri.org/toolbox/ambit/), ChemSpider (https://www.chemspider.com/about), and ChEMBL (https://www.ebi.ac.uk/chembl/). Additionally, software tools such as SwissADME (Swiss Institute of Bioinformatics; http://www.swissadme.ch/index.php) and Molinspiration (http://www.molinspiration.com/) allow users to generate physicochemical properties for chemicals and classify them categorically (Madden, Enoch, Paini & Cronin, 2020). The integration of bioinformatics software and databases enables the early identification of potential toxicity during product development. By identifying problematic candidates early in the process, only successful products progress to advanced stages, thereby reducing the number of chemicals tested on animals. Ultimately, this approach contributes to a significant reduction in animal testing while improving research efficiency and ethical standards.

Bioinformatics Approaches in Plant Biotechnology

Bioinformatics, a field that integrates information technology with biological data management, plays a crucial role in the exploration of plant genomes and the enhancement of economically significant plant traits (Singh, Singh, Chand & Kushwaha, 2011). Initially, genome sequencing efforts were focused on model organisms; however, advancements in high-throughput experimental techniques have since enabled the generation of vast amounts of biological data. In recent years, bioinformatics has evolved to not only facilitate the storage and processing of this data but also to support hypothesis development, result interpretation, and statistical analysis.

In plant biotechnology, the application of bioinformatics tools, along with the analysis and visualization of complex datasets, has contributed to a deeper understanding of biological systems.

This, in turn, plays a vital role in improving essential economic traits such as yield, plant quality, and disease resistance.

Next-Generation Sequencing (NGS)

Next-generation sequencing (NGS) technology has enabled the generation of vast amounts of genetic data from plants, leading to the establishment of numerous databases such as EMBL, GenBank, Phytozome, and Plant GBD for storing and managing this information as raw data (Stoesser et al., 2001; Duvick et al., 2007; Benson, Karsch-Mizrachi, Lipman, Ostell & Sayers, 2009; Goodstein et al., 2012). However, these databases primarily handle genetic information in its raw form, without integrating phenotypic or variant data. In contrast, tools like KnetMiner serve as knowledge network mining platforms capable of searching literature and various databases to connect genetic, omic, and phenotypic information about plants (https://knetminer.com/). By doing so, they facilitate the creation of biological narratives linking genetic data to phenotypic traits.

RNA Sequencing (RNA-seq)

In recent years, bioinformatics applications have extended beyond genome sequencing to RNA sequencing (RNA-seq), where gene products are both sequenced and quantified. RNA-seq, powered by NGS technology, provides detailed insights into RNA sequences and their quantities within a given sample (Wang, Gerstein & Snyder, 2009; Rao et al., 2019). As a foundation of the transcriptomics domain, RNA-seq combined with bioinformatics enables the analysis of gene expression levels, functions, structures, and regulatory mechanisms in specific tissues or cells during functional or developmental stages (Tyagi, Singh, Mathur, Singh & Ranjan, 2022). The resulting data offer crucial insights into gene expression profiles during growth, development, and responses to environmental stressors or diseases (Aranday-Cortes et al., 2012; Gomez-Casati et al., 2018).

RNA-seq studies have been extensively conducted on various model plants, including *Arabidopsis thaliana* (Zhang, Xu, Shang & Wang, 2019), *Rehmannia glutinosa* (Ma et al., 2021), *Oryza sativa* (Li, Bai, Wu, Deng, & Zhou, 2012; Pradhan, Pandit, Nayak, Behera & Mohapatra, 2019; Yang et al., 2021), *Asarum sieboldii* (Chen et al., 2021), *Zea mays* (Xu B et al., 2014; Liu et al., 2019; Xu et al., 2021) , *Polygonum cuspidatum* (Wang et al., 2021) and *Calotropis gigantea* (Hoopes et al., 2018). These studies have provided valuable information on gene expression patterns under various biological and environmental conditions.

RNA-seq Library Database

Over the years, numerous RNA sequencing libraries have been compiled from plant studies. As of 2021, around 45,000 RNA-seq libraries have been gathered for crops such as soybean, maize, wheat, rice, and cotton (Yu, Zhang, Long, Shu & Zhai, 2022). These libraries are publicly accessible through RNA-seq databases, including the European Nucleotide Archive (ENA, https://www.ebi.ac.uk/ena) at EBI, the Sequence Read Archive (SRA, https://www.ncbi.nlm.nih.gov/sra) at NCBI, the Genome Sequence Archive (GSA, https://ngdc.cncb.ac.cn/gsa) at BIG Data, and the Sequence Read Archive (DDBJ-DRA, https://www.ddbj.nig.ac.jp/dra/index-e.html) at DDBJ (Liu et al., 2023). However, some of these databases only provide processed data, limiting their use for comparative analysis across studies. The Plant Public RNA-seq Database (PPDR; https://plantrnadb.com/) and PlantDB (Exner, Hirsch-Hoffmann, Gruissem & Hennig, 2008) combine RNA-seq libraries from public resources like NCBI, DDBJ, SRA, and ENA, offering a more user-friendly experience for researchers (Yu, Zhang, Long, Shu & Zhai, 2022).

Alternative Splicing Database

It is now widely recognized that alternative splicing allows a single gene to produce multiple mRNA transcripts with distinct functions, each potentially translating into a different protein. This

process enhances the functional diversity of genes at the post-transcriptional level (Zhao, 2019). RNA-seq analysis enables the detection of all transcripts, including splice junction regions (Tyagi, Singh, Mathur, Singh & Ranjan, 2022). Several resources focus on model plants like Arabidopsis, such as The Arabidopsis Information Resource (TAIR) (Poole, 2007) and the Arabidopsis Splicing-Related Genes (ASRG) (Wang & Brendel, 2004; Nejat, Ramalingam & Mantri, 2018). Additionally, databases like PlantExp allow users to reanalyze and explore plant gene expressions and alternative splicing. For example, PlantExp (https://biotec.njau.edu.cn/plantExp/) is a publicly accessible, web-based platform that hosts over 130,000 well-curated RNA-seq datasets from 85 species across 24 different orders. Users can query gene expression and alternative splicing profiles, conduct expression analyses, and investigate the conservation of gene expression across species (Liu et al., 2023).

Metabolomics

As with other "omics" fields, bioinformatics tools play a crucial role in metabolomics. Metabolomics offers insights into the roles of metabolites in plant physiology, development, and responses to both biotic and abiotic stresses, as well as helps identify metabolites linked to specific traits or functions (Manicham et al., 2023). Plant metabolomics involves comprehensive analyses of various metabolites, such as sugars, amino acids, organic acids, secondary metabolites (e.g., alkaloids and flavonoids), lipids, and others, in plant tissues and cells. The software used in metabolomics analyses must be capable of processing spectral data, detecting metabolites expressed at significant levels through statistical analysis, and identifying metabolites by referencing metabolite databases. Additionally, these tools must support the creation and visualization of molecular interaction networks through bioinformatics analyses and facilitate the integration and analysis of multi-omics data. Examples of such software include MetFlow (Shen & Zhu, 2019), Mzmine3 (Schmid et al., 2023), MS-DIAL 4.0

(Tsugawa et al., 2020), MetaboAnalyst 6.0 (Pang et al., 2024; https://www.metaboanalyst.ca/), and Omicsnet (Zhou, Pang, Lu, Ewald & Xia, 2022; Chen, Li & Xu, 2022).

Metabolomics Databases

Metabolomics data are shared across various databases. For example, PMhub (Tian et al., 2023) includes chemical data for over 188,000 plant metabolites from various sources and approximately 1.5 million high-resolution tandem mass spectrometry (HRMS/MS) spectra related to these metabolites. The Plant Metabolite Database (PMDB) contains 72,308 electrospray-ionization (ESI)-MSn spectra from a publicly available mass spectrometry library. RefMetaPlant provides reference metabolomes for 153 plant species (Shi et al., 2024).

MetaboLights (https://www.ebi.ac.uk/metabolights/) is a comprehensive metabolomics repository that includes metabolite structures, reference spectra, and associated biological data such as roles, locations, and concentrations. It also features experimental data from metabolic studies. MeT-RO (https://www.rothamsted.ac.uk/facilities-and-resources/metabolomics-rothamsted-met-ro) provides a high-throughput plant metabolomics facility accessible to both internal and external users, offering a variety of resources for plant and microbial metabolomics.

In addition to general plant databases, some species-specific databases have been developed. For example, the Metabolome Tomato Database (MoTo DB) focuses on liquid chromatography and mass spectrometry (LC-MS)-based metabolomics of tomato fruit (*Solanum lycopersicum*) (Grennan, 2009). The Plant Metabolomics (PM) database serves as a functional genomics tool with extensive data on Arabidopsis mutants across various metabolomic platforms to study the roles of Arabidopsis thaliana genes (Bais, Moon-Quanbeck, Nikolau & Dickerson, 2012). There are also bioinformatics tools that integrate detected metabolites with

metabolic pathways. For instance, PlantCyc (https://plantcyc.org/) provides information on metabolic pathways found in over 500 plant species, with a database covering 155 species/taxon-specific pathways. The MetaCyc database (https://metacyc.org/) includes 3,153 pathways, 19,020 reactions, and 19,372 metabolites, while the BioCyc database (https://biocyc.org/) contains 20,052 pathways/genomes.

Resistant Plants Through Gene Editing Tools

The complete genome sequencing of plants, facilitated by bioinformatics tools, has greatly advanced our understanding of plant-pathogen interactions and the mechanisms behind various diseases. As a result, genetically modified crops resistant to pests and diseases have been developed (Koltai & Volpin, 2003). A prominent example of this is the creation of insect-resistant plants. By sequencing the bacterial genome and identifying genes that enhance soil fertility and protect plants from pests, many plants have been engineered for insect resistance. For instance, genes from the bacterium *Bacillus thuringiensis* have been inserted into several plants, making them resistant to insects.

In recent years, CRISPR/Cas9 technology has gained widespread use in developing plants resistant to various pathogens. For this purpose, guide RNAs (gRNAs) for the Cas9 system are designed using bioinformatics tools (Hu, Scheben & Edwards, 2018). Using this technology, many pathogen-resistant plant varieties have been created, including tomato resistant to the powdery mildew pathogen *Oidium neolycopersici*, soybean resistant to *E. diffusa* powdery mildew, rice resistant to the fungal pathogen *M. oryzae*, citrus resistant to *Xanthomonas citri* (which causes canker in citrus fruits), and banana plants resistant to the endogenous banana streak virus (eBSV) (Wang et al., 2016; Jia, Orbovic, Jones & Wang, 2016; Nekrasov et al., 2017; Ma et al., 2018; Bui et al., 2023).

Plant Breeding

Bioinformatics applications in agriculture, aided by emerging technologies, offer an interdisciplinary approach to addressing global challenges such as climate change, water and land scarcity, and the growing need to feed an expanding population (Moose & Mumm, 2008; Godfray et al., 2010; Tilman, Balzer, Hill & Befort, 2011; Watson et al., 2018). Advances in next-generation sequencing (NGS) technologies have made techniques like genotyping-by-sequencing (GBS) and allele discovery particularly useful for species with large genomes and high polymorphism. Data from these methods have helped improve crops through genome-wide association studies (GWAS), identifying quantitative trait loci (QTLs) and allelic variations. By testing multiple genetic variants across the genome to explore genotype-phenotype relationships, GWAS aids in crop breeding by supporting genomic selection and genetic modification (Tan et al., 2022).

Numerous databases have been developed to store, analyze, and share the vast biological data generated by these methods. Some of these databases include Grain Genes (https://wheat.pw.usda.gov/GG3/), Gramene (https://www.gramene.org/), and WheatIS (https://www.wheatis.org/) (Matthews, Carollo, Lazo & Anderson, 2003; Scheben, Batey & Edwards, 2018; Ware, Naithani & Tello-Ruiz, 2020). Additionally, the MetaQTL bioinformatics tool (https://bioinformatics.org/mqtl/wiki/) has been developed to integrate data from gene mapping experiments (e.g., QTLs, candidate genes). MetaQTL is a modular library and set of programs written in Java that offers various functions for formatting, analyzing, and visualizing data.

Furthermore, machine learning techniques, which rely on algorithms that detect patterns in data, have significant advantages for analyzing large datasets (Libbrecht & Nobel, 2015). Machine learning is effectively used to study the relationships between traits and underlying molecular mechanisms.

Conclusions

Bioinformatics has become a cornerstone of modern agricultural biotechnology, revolutionizing the way we approach plant and animal research. With advances in genomic technologies like next-generation sequencing (NGS) and genome-wide association studies (GWAS), bioinformatics has significantly enhanced our ability to analyze complex genetic data, identify important traits, and develop crops and livestock with improved characteristics. The integration of bioinformatics tools has not only streamlined data processing but also enabled more accurate predictions, accelerated gene discovery, and provided new insights into the genetic basis of key agricultural traits such as disease resistance, yield, and nutritional content. As bioinformatics continues to evolve, it will undoubtedly play an even more critical role in addressing global challenges, such as climate change and food security, by enabling more efficient and targeted advancements in both plant and animal biotechnology. The continued development and application of bioinformatics will shape the future of agriculture, leading to more sustainable and productive agricultural practices worldwide.

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CHAPTER XIII

Effects of High and Low Doses of Glutamine on the Germination of Rocket Under Salt Stress Conditions

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Introduction

Rocket belongs to the *Brassicaceae* family, one of the families that includes widespread cultivated plants. This family includes many cultivated plants that are interesting in terms of morphology and variety of uses, and it is thought that these plants were first consumed as greens because of their pungent taste (Daun, 2011). The Brassica family is concentrated in temperate regions and the plants have maximum diversity in the Mediterranean and North African regions (Kimber & McGregor, 1995). Rocket has a well-developed root system and is drought tolerant. Thanks to its strong structure, it has spread to all inhabited regions (Garg & Sharma,

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2014). In addition to its fresh and processed consumption, it is also used as an oil and fodder plant (Hall, Jobling, & Rogers, 2013; Garg & Sharma, 2014). Rocket is a very important vegetable species due to its important phytochemical content, as well as its anticancer, antioxidant, antiangiogenesis, and anti-inflammatory properties (Duru & al., 2022).

Salt stress is one of the important abiotic stresses that negatively affects different stages of plant development such as germination, cell division and elongation, photosynthesis, vegetative development of the plant, productivity and product quality. More than 20% of the arable land in the world is negatively affected by salt stress (Trușcă & al., 2023; Arora, 2019). As salt stress is increasing day by day, there is a need to focus more on stress coping methods from the first stage of plant development.

External applications to the plant are one of the methods to cope with salt stress. Glutamine (Gln) is the first amino acid synthesised during nitrogen (N) assimilation in plants and acts as an N-donor in many chemical reactions. It induces somatic embryogenesis and shoot organogenesis in plants. Exogenous Gln has been implicated in the activation of stress and defence responses in plants (Lee & al., 2023). The aim of this study was to determine the effects of Gln on salt stress tolerance during the germination stage of arugula by applying low and high doses of external Gln.

Materials and Methods

Plant materials

The research was carried out in the Organic Agriculture Laboratory of Akdeniz University Vocational School of Technical Sciences. Two rocket varieties (Doruk, Izmir) used in the study were purchased from a commercial company.

Methods

Before applying Gln to the seeds, surface sterilisation was carried out. After washing the seeds with tap water, they were kept in 10% sodium hypochlorite solution with continuous mixing for 10 minutes. They were then kept in 70% ethyl alcohol for 1 minute and then rinsed with sterile distilled water three times for 5 minutes each to complete the sterilisation process. The sterilised seeds were kept in low doses of 1, 2 and 3 mM Gln and high doses of 10, 20 and 30 mM Gln at room temperature for 24 hours. The control seeds were kept in sterile distilled water.

In order to determine the effect of Gln on germination under salt stress conditions, a previous study (Nasircilar & Ulukapi 2023) was taken as a basis and a 150 mM NaCl concentration was used. Control and Gln-treated seeds were placed in 90 x 15 mm Petri dishes with two layers of sterile blotting paper. A total of 100 seeds were used for each treatment. The petri dishes were placed in a climate chamber at 24±1ºC and 16/8 h photoperiod and the germination experiment was continued for 14 days according to ISTA rules (ISTA 1985). The control group was watered with distilled water and the salt-stressed seeds were watered with a solution containing 150 mM NaCl. The emergence of the radicle from the testa was taken as germination and germination counts were made daily.

At the end of the experiment, germination percentage (GP), mean germination time (MGT), coefficient of germination rate (CVG) and germination index (GI) were calculated as germination parameters. The formulae used to calculate germination parameters are given below.

GP (%) = Number of germinated seeds / Total number of seeds X 100 (Gosh et al., 2014)

MGT: Σ Dn / Σn D = days counted from the beginning of the test, n = number of seeds germinated on day D (Ellis & Roberts, 1981; Sivritepe, 2012).

CVG: N1+N2+....+Nx/100xN1T1+.....+NxTx (Kotowski, 1926) T: number of days corresponding to N, N: number of seeds germinated each day
GI: (14xn1) + (13xn2) +.....+ (1xn14) n1, n2,..., n14: Number of seeds germinated on the first, second and subsequent days until day 14 (Benech et al., 1991).

Statistical analysis

The germination experiment was carried out in 4 replicates with 25 seeds in each replicate. The analysis of variance was carried out using the MINITAB 21 package program and the Excell program was used to visualise the germination parameters.

Results and Discussion

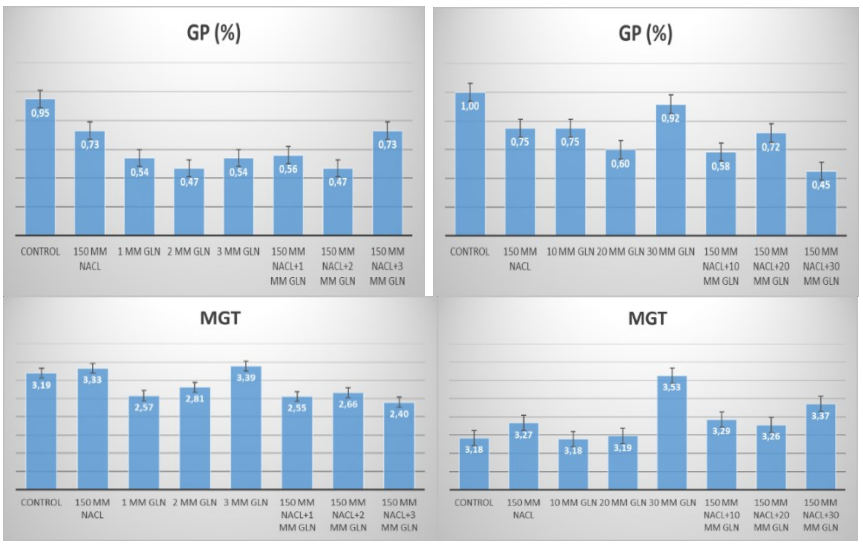
In this study, the effect of Gln application on germination parameters of rocket seeds exposed to salt stress at the germination stage was investigated. According to the results of variation analysis presented in Table 1, cultivar, which is one of the sources of variation, had a statistically significant effect on MGT at 0.05 level and on CVG at 0.01 level. When all the other parameters were analysed, the interaction between cultivar, treatment and variety treatment on GP, MGT, CVG, GI were found to be statistically significant at 0.001 level.

Table 1. Variance analysis result with respect to the germination parameters of rocket cultivars.

Variation source	df	GP (%)	MGT (day)	CVG	GI
C	1	***	*	**	***
T	15	***	***	***	***
C*T	15	***	***	***	***

C: Cultivar, T: Treatment, * $p<0.05$, **$p<0.01$, ns: not significant ***$p<0.001$

Recent studies have shown that Gln, a proteinogenic amino acid involved in different pathways for the synthesis of many important molecules, also acts as a signalling molecule that induces different transcription factors responsible for plant defence against abiotic stress (Miranda & al., 2017; Han & al., 2022). However, this effect varies between plant species and even between cultivars. In this study, two different varieties of the same species showed different responses. It was found that both high and low doses of Gln did not increase or even decrease GP in cultivar Doruk (Figure 1). However, especially the low dose of Gln (3 mM Gln: 2.40) reduced the germination time under salt stress conditions. Similarly, low dose of Gln had a positive effect on CGV (3 mM: 41.71) but not on GI under salt stress. In this study, high levels of exogenous glutamine had a negative effect on germination in Doruk and the same negative effect on plant development was observed in poplars (Han & al., 2022).



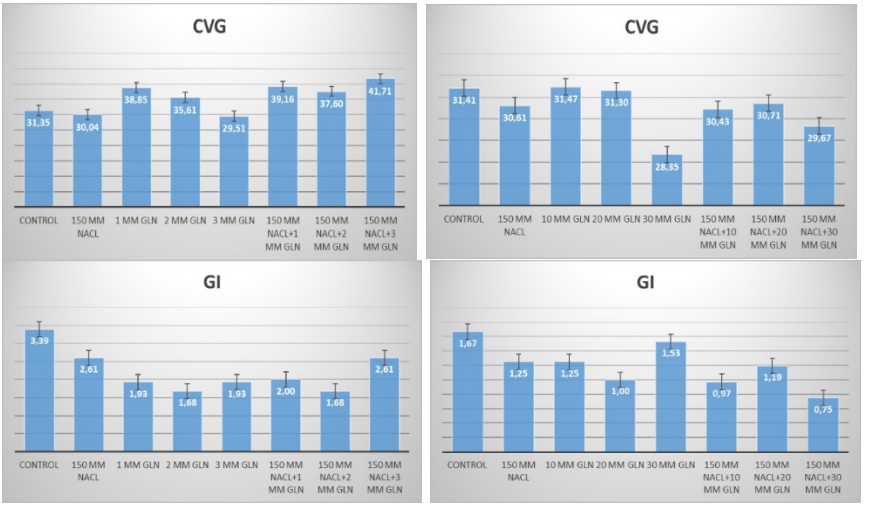


Figure1. *Changes in germination parameters of Doruk rocket treated with low and high doses of Gln*

In cultivar Izmir, germination percentage increased from 80% under salt stress to 90% with 1 mM Gln applied under the same conditions (Figure 2). Higher doses had a decreasing effect on GP. Similarly, 1 mM Gln applied under salt stress decreased MGT (2.29), increased CVG (43.69) and GI (3.21). Consistent with this study, the inhibitory effect of salt stress on seed germination was significantly reduced by Gln application in the study conducted on *Allium cepa* (Çavuşoğlu & al., 2020). A study on carrots also showed that different varieties responded differently to externally applied Gln. Glutamine application (1 mM) under salt stress was found to have a positive effect on germination only in the orange carrot variety (Üstüner & al., 2023).

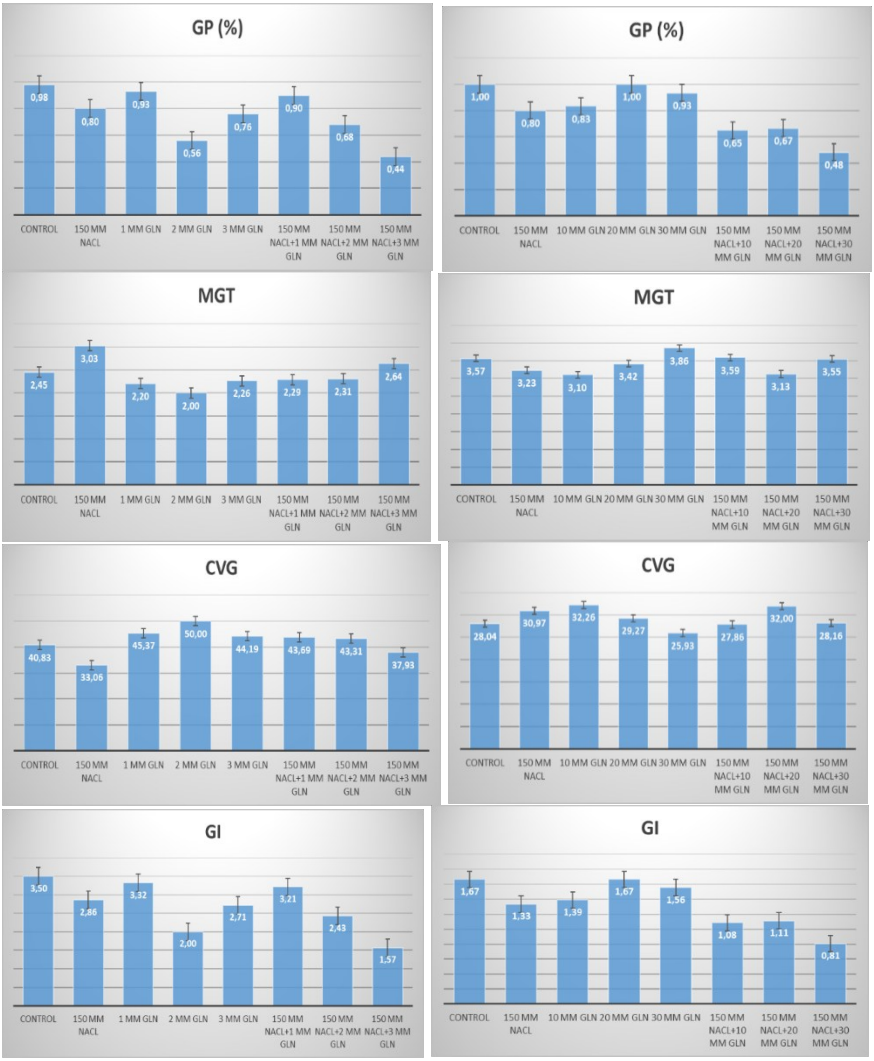


Figure 2. *Changes in germination parameters of İzmir rocket treated with low and high doses of Gln*

Conclusion

As a result, low dose Gln application provided more favourable results on the germination parameters of rocket seeds

under salinity conditions. However, these data refer only to germination parameters and it will be useful to determine the most appropriate application dose according to needs by studying the advanced stages of the plant.

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CHAPTER IX

How Different Doses of Glutamine Priming Affect the Germination Parametres 0f Garden Cress (*Lepidium Sativum* L.) Under Salt Stress?

Ayse Gul NASIRCILAR[1]
Kamile ULUKAPI[2]

Introduction

Soil salinization, one of the most important abiotic stress factors that threaten agricultural yield and ecological security on a global scale, limits plant growth and productivity. Plants can survive under salt-stress conditions only if they effectively cope with the negative effects of salt stress (Zhou & al., 2024). One of the main problems in saline areas is low seed germination and seedling emergence. This is because the final plant density achieved is directly related to the germination rate and strength of the seed

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(Habibi & Abdoli, 2013). Priming technology, which involves soaking seeds in water or solutions containing natural or synthetic agents prior to planting, is a method used to induce stress tolerance in seeds (Moulick & al., 2018). Seed priming has been used in various crops as a cost-effective alternative method to confer resistance to biotic and abiotic stress conditions without introducing genetic or transgenic changes in plants (Moulick & al., 2016).

Amino acids, which play an important role in plant growth and development, are biostimulants with potential for use in organic agriculture. These properties make them an alternative source for sustainable agriculture (Abdelkader & al., 2023). Amino acids, precursor molecules for synthesizing nitrogenous compounds, especially proteins, also play an important role in stress tolerance. In recent years, studies have shown that Gln is one of the amino acids contributing to stress tolerance (Nasırcılar & Ulukapı, 2023).

Garden cress (*Lepidium sativum*) is a medically important vegetable of the *Brassicaceae* family. In addition to its low nutritional requirements and its rapid growth, it is recommended by the OECD as a model plant to determine the effects of environmental stresses such as heavy metal stress (Nouri & Haddioui, 2021; OECD, 1994). Only a few seed priming studies have been carried out on garden cress. In the study investigating the effect of hydropriming on the germination parameters of cress, it was found that this application shortened the germination period and increased the yield of uniform seedlings (Noorhosseini & al., 2018). Among the different priming treatments applied to cress seeds exposed to arsenic stress, the best results on germination and seedling growth parameters were obtained with the gibberellic acid treatment (Nouri & Haddioui, 2021). Morover salicylic acid priming was found to have a positive effect on the germination of cress under salt stress (Habibi & Abdoli, 2013). The aim of this study was to determine the effects of high and low doses of Gln priming under saline conditions on germination parameters of garden cress, a medically important vegetable, under salt stress.

Materials and Methods

Materials

Two different cress cultivars (BT and Gülfem) purchased from a commercial company were used in the research. Salt stress was created with 150 mM NaCl and low (1, 2, 3 mM) and high doses (10, 20, 30 mM) of Gln were used for priming. The study was carried out in the controlled climate room of Akdeniz University Vocational School of Technical Sciences, Department of Plant and Animal Production.

Priming application

The seeds were kept in 10% sodium hypochlorite and 70% alcohol for 10 and 1 minute respectively with continuously stirring for surface sterilization. The seeds were rinsed with sterile distilled water for 5 minutes 3 times and transferred to jars containing low (1, 2, 3 mM) and high doses (10, 20, 30 mM) of Gln. Gln priming was carried out by keeping the seeds at room temperature for 24 hours. Control seeds were kept in sterile distilled water under the same conditions.

Germination parameters

Gln applied seeds were transferred to petri plates (90x15) containing sterile blotting paper. In the experimental group, each petri was watered with equal amounts of salt water to create salt stress, while only water was used in the control group. The seeds were stored in a climate chamber with a temperature of 24±1ºC and a photoperiod of 16/8 hours (light/dark) for 14 days. Trials were carried out in 4 replicates with 25 seeds each. According to ISTA (1985) rules, the trial continued for 14 days and the germinated seeds were counted daily. Germination parameters such as germination percentage (GP), mean germination time (MGT), germination rate coefficient (CVG) and germination index (GI) were calculated according to the formulas below.

GP (%) = Number of germinated seeds / Total number of seeds X 100 (Gosh & al., 2014**)**

MGT: Σ Dn / Σn D = days counted from the beginning of the test, n = number of seeds germinated on day D (Ellis & Roberts, 1981; Sivritepe, 2012).

CVG: N1+N2+….+Nx/100xN1T1+……+NxTx (Kotowski, 1926) T: number of days corresponding to N, N: number of seed germinated each day

GI: (14xn1) + (13xn2) +…..+ (1xn14) n1, n2,…, n14: number of germinated seeds on the first, second and subsequent days until the 14th; 14, 13…., and 1 are weights given to the number of germinated seeds on the first, second and subsequent days respectively (Benech & al., 1991).

Statistical analysis

The statistical analysis of the data was determined by variance analysis in the Minitab 21 package program. The visualization of the studied parameters was done with the Excell program.

Results and Discussion

Gln, which has an important role in the synthesis of nitrogenous compounds such as amino acids and nucleotides in all organisms, functions as a metabolic fuel (Kan & al., 2015).

In this study, which aimed to determine the effect of Gln on germination under both normal and salt stress conditions, according to the results of the analysis of variance performed to determine the effect of application and variety on germination parameters, it was determined that both variety and all priming applications had a statistically significant effect on all germination parameters at the level of $p<0.001$ (Table 1).

Table 1. Variance analysis result with respect to the germination parameters of cress cultivars

Variation source	df	GP (%)	MGT (day)	CVG	GI
C	1	***	***	***	***
A	15	***	***	***	***
C*A	15	***	***	***	***

C: Cultivar, A: Application, GP: Germination Percentage, MGT: Mean Germination Time, CVG: Coefficient of Germination Velocity, GI: Germination Index; ***$p<0.001$

Salt stress negatively affected germination parameters in both cultivars (Figure 1, Figure 2). In BT cv. GP, which was 93% in control plants, decreased to 47% in salt stress. In salt conditions, MGT increased relatively and GI decreased. GI value, which was 1.56 in control seeds, decreased to 0.78 under salt stress. Similarly, in Gülfem cv. GP, which was 100% in control, decreased to 58% in salt stress and germination time was prolonged.

Gln priming under normal conditions did not stimulate the GP in both cultivars at both high and low concentrations. Especially in BT cv., when applied at high doses, it caused a significant decrease in GP. The germination percentage, which was 93% in the control, decreased to 72% with 20 mM Gln priming (Figure 1). A study supporting these results was carried out on rice seedlings. When 0.1-10 mM Gln was added to rice seedlings grown in hydroponic conditions as a nitrogen source, it was found that low doses of Gln (0.1mM, 0.5 mM) supported root and shoot development in the plant, whereas the addition of 1, 2.5, 5 or 10 mM Gln inhibited the growth of rice seedlings (Kan & al., 2015). It is therefore concluded that even the low dose of Gln used in this study was relatively high.

Although the application of Gln did not increase the GP under normal conditions, it had a promoting effect on some germination parameters in current study. Both high and low doses of Gln slightly shortened the MGT of BT cv. At 1 mM Gln the

germination time was reduced to the lowest value of 3.42 days. In connection with this, the CVG value also increased to the highest value of 30.83 with 1 mM application.

In a study where onion seeds were primed with 10 different amino acids, the best improvement in growth parameters was obtained in seeds pre-treated with Gln. In particular, exogenous Gln application increased the vigour index, resulting in an increase in the rate of obtaining normal seedlings (Abdelkader & al., 2023). Gln priming was performed on 5 different carrot varieties and although Gln had a positive effect on germination parameters in orange and yellow carrot cultivars, the highest germination was obtained in purple carrot cultivar in control seeds (Üstüner & al., 2023).

These results suggest that the effect of Gln on germination and growth parametres varies between plant species and even between genotypes. In addition, the dose of application is also very important and appropriate doses should be determined in studies.

Under salt stress conditions, Gln priming had a positive effect on germination parameters for both cultivars, unlike normal conditions (Figure 1, Figure 2). This germination-promoting effect was obtained from both low and high dose applications.

In BT cv., as a result of low dose Gln application, the germination percentage, which was 48% in saline conditions, increased with all three application doses. The highest GP was obtained with 3 mM Gln and increased to 69%. In high dose applications, the GP showed a gradual increase depending on the dose, and the rate, which was 47% under salt stress, became 63% with 30 mM Gln priming. Under saline conditions, Gln priming slightly decreased the MGT. This was especially achieved with low dose application, and the mean germination time, which was 3.46 days under salt stress, was shortened to 2.40 days with 3 mM Gln. Similarly, the best results were obtained using the low dose of 3 mM

for CVG and GI values. CVG increased from 27.34 to 41.67 and GI from 1.36 to 1.96.

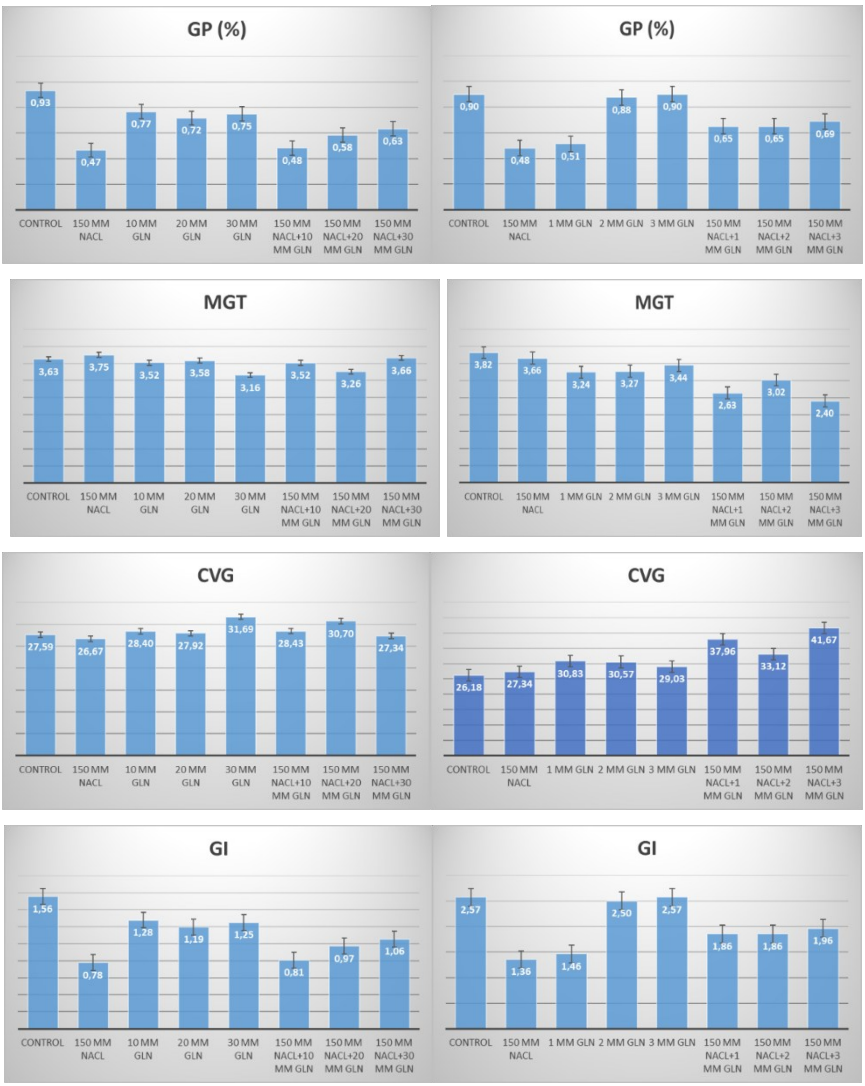

Figure 1. *Effects of high and low dose glutamine priming on germination parameters in BT cv. under normal and saline conditions*

In Gülfem vc., Gln application had an increasing effect on germination rate under saline conditions. All Gln doses increased the GP under salt stress. The highest germination percentage was determined as 81% with 1 mM Gln application. Similarly, 1 mM Gln had a positive effect on germination parameters in the orange carrot cultivar under salt stress conditions (Üstüner & al., 2023). At high dose, the best result was obtained from 10 mM Gln and the germination rate, which was 58 % under salt conditions, increased to 78 % with this dose in current research. It was observed that high doses of Gln were more effective than low doses in reducing the MGT. 10 mM Gln reduced mean germination time from 3.77 days to 3.51 days under saline conditions. Similarly, the most significant increase in GI value was obtained with 10 mM application, and the value increased from 0.97 to 1.31.

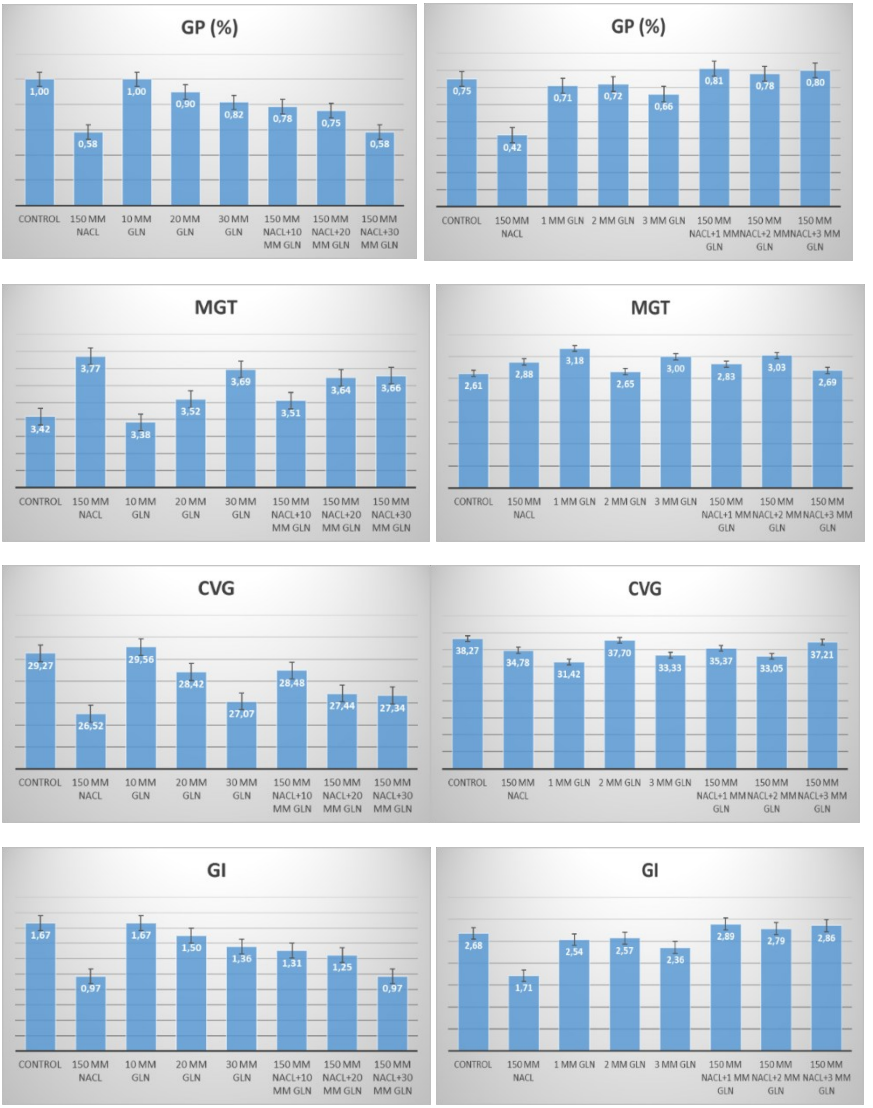

Figure 2. *Effects of high and low dose glutamine priming on germination parameters in Gülfem cv. under normal and saline condition.*

Conclusion

As a result, especially under salt stress conditions, both high and low doses of Gln priming had a stimulating effect on different germination parameters in garden cress, reversing the negative effects of stress. Although it is not effective in increasing the germination percentage under normal conditions, it is recommended to determine its effects using doses below 1 mM due to its stimulating effect on other parameters.

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