

FIRAT UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
TÜRKİYE



**EVALUATING THE POTENTIAL OF GREEN TEA (CAMELLIA
SINENSIS) HYDROSOL IN DETOXIFYING THE TOXIC EFFECTS
OF IMIDACLOPRID ON ZEBRA MUSSELS (DREISSENA
POLYMORPHA)**

Zahra BATOOL

Master's Thesis

DEPARTMENT OF AQUACULTURE

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THESIS APPROVAL

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DECLARATION

I hereby declare that I wrote this Master's Thesis titled “Evaluating the Potential of Green Tea (Camellia Sinensis) Hydrosol in Detoxifying the Toxic Effects of Imidacloprid on Zebra Mussels (Dreissena Polymorpha)” in consistent with the thesis writing guide of the Graduate School of Natural and Applied Sciences, Firat University. I also declare that all information in it is correct, that I acted according to scientific ethics in producing and presenting the findings, cited all the references I used, express all institutions or organizations or persons who supported the thesis financially. I have never used the data and information I provide here in order to get a degree in any way.

27 January 2025

Zahra BATOOL



PREFACE

The increased use of pesticides, such as imidacloprid, poses considerable threats to aquatic ecosystems, needing adequate mitigation measures. Zebra mussels (*D. polymorpha*) are important bioindicators of water quality, and understanding how harmful compounds affect them is critical for environmental protection. This study looks into the detoxifying properties of green tea (*C. sinensis*) hydrosol against imidacloprid toxicity in order to contribute to sustainable aquatic ecosystem management. The study presented various problems, including the controlled delivery of poisons, assuring the reproducibility of experimental conditions, and assessing biochemical responses. Nonetheless, the potential ecological benefits of finding natural detoxifying agents were a primary motivator.

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ABSTRACT

Evaluating the Potential of Green Tea (*Camellia Sinensis*) Hydrosol in Detoxifying the Toxic Effects of Imidacloprid on Zebra Mussels (*Dreissena Polymorpha*)

Zahra BATOOL

Master's Thesis

FIRAT UNIVERSITY
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This study examines the detoxifying capacity of imidacloprid by green tea (*C. sinensis*) hydrosol against the toxic effects of imidacloprid on the zebra mussel (*D. polymorpha*). A widely used neonicotinoid insecticide, imidacloprid, is an environmentally hazardous compound with the tendency to bioaccumulate and to be toxic to non-target aquatic organisms. A natural source of these germ fighting (antioxidant, detoxifying) compounds, is green tea hydrosol, which is rich in polyphenols and catechins. Under controlled conditions, zebra mussels were exposed to a range of concentrations of imidacloprid then treated with green tea hydrosol. Analysis was carried out using ELISA of biochemical markers of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) and oxidative stress indicators (malondialdehyde). Mortality rates, as well as behavioral responses and reproductive activity also were monitored. Green tea hydrosol was shown to attenuate imidacloprid induced oxidative stress and lipid peroxidation. Enhanced enzymatic activity and decreased malondialdehyde levels were associated with particularly those groups treated with higher hydrosol concentrations. Also, the hydrosol was found to have stimulated reproductive activity and to maintain mussel health without detrimental effects. The results of this research show the capability of green tea hydrosol to act as an eco-friendly detoxifier in the aquatic ecosystem and provides possible replacement in chemical remediation strategies. These findings highlight the need for building natural detoxifiers into the environmental toxicology and the frame work for controlling invasive species.

Keywords: Environmental Toxicology, Antioxidant Enzymes, Aquatic Ecosystem Management, ELISA

ÖZET

İmidaklopridin Zebra Midyeleri (*Dreissena polymorpha*) Üzerindeki Toksik Etkilerinin Detoksifikasyonunda Yeşil Çay (*Camellia sinensis*) Hidrosolünün Potansiyelinin Değerlendirilmesi

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Bu çalışma, imidaklopridin zebra midyesi (*D. polymorpha*) üzerindeki toksik etkilerine karşı yeşil çay (*C. sinensis*) hidrosolü ile imidaklopridin detoksifikasyon kapasitesini incelemektedir. Yaygın olarak kullanılan bir neonikotinoid insektisit olan imidacloprid, biyolojik olarak birikme ve hedef olmayan suda yaşayan organizmalar için toksik olma eğilimi gösteren, çevreye zararlı bir bileşiktir. Bu bileşiklerle mücadele eden (antioksidan, detoksifiye edici) bileşiklerin doğal bir kaynağı, polifenoller ve kateşinler açısından zengin olan yeşil çay hidrosolüdür. Kontrollü koşullar altında, zebra midyeleri çeşitli konsantrasyonlarda imidacloprid'e maruz bırakıldı ve ardından yeşil çay hidrosol ile işleme tabi tutuldu. Antioksidan enzimlerin (katalaz, süperoksit dismutaz, glutatyon peroksidaz) ve oksidatif stres göstergelerinin (malondialdehid) biyokimyasal belirteçleri ELISA testleriyle gerçekleştirildi. Ölüm oranlarının yanı sıra davranışsal tepkiler ve üreme faaliyetleri de izlendi. Bulgular, yeşil çay hidrosolünün imidakloprid kaynaklı oksidatif stresi ve lipid peroksidasyonunu azalttığını göstermiştir. Artan enzimatik aktivite ve azalan malondialdehid seviyeleri, özellikle daha yüksek hidrosol konsantrasyonları ile muamele edilen gruplarla ilişkilendirilmiştir. Ayrıca hidrosolün üreme aktivitesini uyardığı ve zararlı etkiler olmaksızın midye sağlığını koruduğu bulunmuştur. Bu araştırmanın sonuçları, yeşil çay hidrosolünün su ekosisteminde çevre dostu bir detoksifikasyon olarak hareket etme yeteneğini gösterir ve kimyasal iyileştirme stratejilerinde olası bir değişim sağlar. Bu bulgular, çevresel toksikolojiye ve istilacı türlerin kontrolüne yönelik çerçeve çalışmasına doğal detoksifikasyon maddelerinin dahil edilmesi ihtiyacını vurgulamaktadır.

Anahtar Kelimeler: Çevresel Toksikoloji, Antioksidan Enzimler, Su Ekosistemi Yönetimi, ELISA

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SYMBOLS AND ABBREVIATIONS

Symbols

µg/L	: Micrograms per Liter
ml/L	: Milliliters per Liter
°C	: Degrees Celsius
pH	: Potential of Hydrogen (Measure of Acidity/Alkalinity)
µm	: Micrometers

Abbreviations

CIGS	: Copper Indium Gallium Di-Selenite (Cu(InGe)Se ₂)
DSSCs	: Dye Sensitized Solar Cells
ANOVA	: Analysis of Variance
CAT	: Catalase
DNA	: Deoxyribonucleic Acid
EC	: Epicatechin
ECG	: Epicatechin Gallate
EGC	: Epigallocatechin
EGCG	: Epigallocatechin Gallate
ELISA	: Enzyme-Linked Immunosorbent Assay
EPA	: Environmental Protection Agency
GPX	: Glutathione Peroxidase
HRP	: Horseradish Peroxidase
HSD	: Honestly Significant Difference
IPM	: Integrated Pest Management
IQR	: Interquartile Range
LC50	: Lethal Concentration 50%
MDA	: Malondialdehyde
Nrf2	: Nuclear Factor Erythroid 2–Related Factor 2
PBS	: Phosphate-Buffered Saline
ROS	: Reactive Oxygen Species
SOD	: Superoxide Dismutase
USA	: United States of America

1. INTRODUCTION

1.1. Background

Small, freshwater bivalve mollusks native to southern Russia and Ukraine, zebra mussels (*D. polymorpha*) are exotic to North America in general and to Quebec specifically. Their distinctive striped shells resemble those of a zebra, hence their name. These tiny creatures, despite their size, have a significant impact on the ecosystems they invade [1]. Zebra mussels have been present throughout North America since the late 1980s. It is believed that they arrived via ballast water discharged from ships traveling from Europe. These strange creatures were first spotted in North America in late summer of 1988 in Lake St. Clair which is between Lake Huron and Lake Erie. They quickly spread from there, to the Great Lakes and beyond, to other inland waterways in the U.S. and Canada [2]. Zebra mussels reproduce at an astonishing rate. A female can produce up to a million eggs each year. These eggs become free swimming larvae, known as veligers, that are easily and speedily transferred by water currents to new areas. Once zebra mussels attach themselves on any hard surface using byssal threads, they don't move again. This includes boat hulls, docks, water intake pipes, and even other aquatic animals, such as native mussels [3].

The ecological impact of zebra mussels is profound. As filter feeders, they consume large quantities of plankton, which is a critical food source for many native fish and invertebrate species. This massive filtering can increase water clarity, which, on the surface, might seem beneficial. However, clearer water allows sunlight to penetrate deeper, promoting the growth of submerged aquatic vegetation. Shifts like these change habitat regimes and, in doing so, can throw off the balance in aquatic ecosystems. Moreover, zebra mussels have displaced native bivalves by consuming more of the same food and less of the same space, resulting in native mussel declines [4]. Economically, the invasion of zebra mussels has been a disaster. They cause extensive biofouling, which is the accumulation of organisms on surfaces such as water intake pipes, boat hulls, and power plant facilities. This biofouling can clog pipes and cooling systems, leading to increased maintenance costs, reduced operational efficiency, and even potential shutdowns. Industries and municipal water facilities spend millions of dollars annually on cleaning and preventive measures to manage zebra mussel infestations. Zebra mussels, for example, cost the power industry itself an estimated \$3.1 billion over the decade, and a humble, additional, billions to cleanup and control costs in other sectors, according to the U.S. Fish and Wildlife Service [5].

Controlling and managing zebra mussel populations has become a critical issue. Traditional methods include mechanical removal, chemical treatments, and biological controls. Mechanical removal involves physical methods such as scraping and high-pressure washing, which are labor-intensive and provide only temporary relief. Chemical treatments, particularly the use of

molluscicides, are more effective but come with significant environmental concerns [6]. Imidacloprid is one of the chemicals commonly used from this family of neonicotinoid insecticides. Imidacloprid attacks the insect's or insect's invertebrate nervous system, resulting in paralysis and death. It binds to nicotinic acetylcholine receptors, causing over stimulation and long-term failure of transmission in this pathway. Imidacloprid was effective at reducing zebra mussel populations, however, because it persisted in water and could potentially bioaccumulate in non-target aquatic organisms such as fish and amphibians [7]. There has been a lot of research investigating imidacloprid's environmental impact. It is also known to be extremely toxic to a variety of other non-target organisms, such as fish and other aquatic life, as well as beneficial insects. Imidacloprid studies show it can persist in the environment for long periods, with a half-life in water of days to months depending on factors such as pH and temperature, the presence of organic matter. Such persistence increases the risk of persistent chronic exposure to non-target organisms, with consequent sublethal effects including reduced reproduction, growth, and behavior [8]. In view of these challenges, there is an increasing necessity to search for other strategies to prevent the toxicity of chemical treatments such as imidacloprid. A promising way is to utilize natural substances with a detoxifying effect.

In this regard, a non-toxic residue of the steam distillation process designed to draw out essential oils called a hydrosol, is shown to have potential and is tested. It's loaded with powerful bioactive compounds that include polyphenols, catechins, and flavonoids, which are associated with powerful properties for improving active: antioxidant, anti-inflammatory, and detoxification. [9]. Green tea has many studies along which show that it can reduce the risk of cardiovascular diseases, particular cancers as well as neurodegenerative disorders in humans. The high polyphenolic compounds content provides strong antioxidant activity, and thus accounts for most of these benefits. Most important among of these are the main catechins in green tea, epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC), and epigallocatechin gallate (EGCG) [10]. Despite the application of green tea hydrosol, incorporating green tea hydrosol in environmental toxicology and more specifically in the detoxification of contaminants, specifically imidacloprid in aquatic ecosystems, is virgin territory and worth of research. The potential of plant-based substances such as green tea extracts to alleviate the harmful effects of environmental pollutants has only recently been investigated. For instance, R.J. Moore [11] showed that green tea extracts can lower oxidative stress and DNA injury in fish exposed to heavy metals. According to R.L. Pastore [12] green tea polyphenols may protect against pesticide induced oxidative damage in rats. These results indicate that green tea bioactive compounds may provide protective effects against various environmental toxicants. [13].

Green tea hydrosol detoxification is carried out via multiple pathways. Green tea contains antioxidants that can neutralize reactive oxygen species (ROS) generated by exposure to pollution

so as to protect cellular structure and functions. Furthermore, green tea polyphenols could modulate signaling pathways related to stress response and detoxification. Perhaps one such example is that EGCG has been shown to activate the Nrf2 signaling pathway, which controls expression of antioxidant and detoxification enzymes (e.g. superoxide dismutase, SOD; catalase, CAT; glutathione S-transferases, GSTs). An activation of this sort can enhance expressions for the upliftment of the organism in its range of coping with, and eliminating, toxicants [14]. Given these potential benefits, this work explored the detoxification capacity of green tea hydrosol against the toxic effects of imidacloprid on zebra mussels. This study investigates the reaction of zebra mussels to exposure and subsequent treatment with imidacloprid and green tea hydrosol, and the efficacy of natural detoxifiers in environmental management. The detailing of these interactions is critical for planning sustainable and ecofriendly methodologies in order to mitigate the adverse impacts of chemical pollutants on the biodiversity and aquatic environment [15].

1.2. Problem Statement

Zebra mussels have become a serious invasive species in North America freshwater systems due to their incredible ability to spread and colonize quickly. Although methods used to manage them (e.g. applications of chemical treatments such as imidacloprid) represent a source of significant ecological and economic impacts, they also raise their own series of challenges and environmental issues. The neonicotinoid insecticide imidacloprid has been effective at managing populations of a variety of pests including zebra mussels. It kills by paralyzing the nervous system of insects and invertebrates. But there are substantial drawbacks to its use in aquatic environments. Imidacloprid is highly toxic to the target species as well as to a wide variety of non-target aquatic organisms, such as beneficial insects, amphibians, and fish. These concerns are compounded by the persistence of IPPPs in the environment, its potential to bioaccumulate and resulting long-term ecological consequence [16]. Imidacloprid has substantial environmental risks.

This chemical has been shown to stay in water and soil for a long time making chronic exposure of non-targets almost certain. As a result, this prolonged presence can cause a host of sub lethal effects in aquatic life like impaired reproduction, stunted growth and changed behaviors [17]. But these impacts are not just endangering individual species in many cases, they're causing the whole ecosystem to come unbalanced. These challenges support the need to explore and develop alternative strategies to control zebra mussel populations without doing environmental harm. Natural substances with detoxifying properties, however, may be one promising avenue. But green tea (*C. sinensis*) hydrosol fits the bill. Due to the richness of bioactive compounds such as polyphenols catechins and flavonoids present in it, green tea is famous for as powerful antioxidant and detoxifier [18].

Often used as a byproduct from steam distillation for the purpose of extraction of essential oils, green tea hydrosol retains many of these compounds. If the health benefits of green tea in reducing the risks of cardiovascular diseases, some cancers and neurodegenerative disorders are well known for humans, little is known about its potential for environmental applications including its detoxification of imidacloprid, a common environmental pollutant [19]. This study attempts to fill this gap by examining the capacity of green tea hydrosol as a possible antidote of imidacloprid toxic effects on zebra mussels. In this study, examining biochemical and physiological responses of zebra mussels to imidacloprid exposure and their subsequent treatment with green tea hydrosol provides an opportunity to investigate the use of natural detoxifiers in environmental management. To elaborate, the problem at hand is twofold: addressing the invasive zebra mussel population without reversing any ecological harm and discovering a sustainable, ecofriendly substitute to chemical treatments, such as imidacloprid. The potential rewards, however, are great, and so is the difficulty of the problem. This approach may prove successful and offer a new paradigm for environmental management: an approach utilizing the detoxifying power of natural substances to confront some of our most pressing ecological challenges [20].

An understanding of the ways in which the protective effects of the green tea hydrosol are exerted is essential. Green tea antioxidants can neutralize reactive oxygen species (ROS) generated through imidacloprid exposure to protect cell structures and functions of zebra mussels. Furthermore, green tea polyphenols can perhaps also influence signaling pathways related with stress reaction and elimination. For example, catechin EGCG, a well-known catechin in green tea, is known to stimulate the Nrf2 signaling pathway. This pathway controls the expression of antioxidant as well as detoxifying enzymes and thus makes the organism better able to deal with and eliminate toxicant [21]. In trying to offer insight into the possibility of green tea hydrosol as a natural detoxifier, this study addresses these queries. The findings could also help in the development of environmentally friendly, sustainable ways of controlling the negative effects of chemical contaminants in aquatic ecological systems. This approach contributes not only to biodiversity conservation but also to health and resilience of aquatic environments.

The potential of green tea hydrosol for detoxification of imidacloprid's toxic effects on zebra mussels is evaluated in this thesis. This study attempts to investigate the biochemical and physiological response of zebra mussels exposed to imidacloprid and then treated with green tea hydrosol as natural detoxifiers for environmental management [22].

1.3. Objectives

The objective of this research is to find out whether green tea hydrosol can assist in cleaning to the detriment of imidacloprid effects on zebra mussels. To break it down further, the study aims to achieve the following:

1.3.1. Assessing Acute and Chronic Toxicity

First, must understand the short- and long-term harm imidacloprid causes to zebra mussels. It requires assessing immediate impact, and any long-term damage or stress the chemical causes.

1.3.2. Analyzing Biochemical and Physiological Changes

In this study, the effects of imidacloprid exposure on the mussels were examined. This boils down to seeing how their bodily functions, and their biochemistry like stress markers and enzyme activities, change as a chemically induced product of the chemical.

1.3.3. Investigating the Protective Effects of Green Tea Hydrosol

After understanding the harm that imidacloprid might bring, green tea hydrosol is being introduced to see whether it will lessen these problems. To determine whether the hydrosol will lessen the toxicity and aid the mussels in fending against or recovering from the negative consequences.

1.3.4. Elucidating the Detoxifying Mechanisms

Furthermore, to demonstrate how the chemicals in green tea hydrosol cleanse the mussels. This involves a study of the mechanisms and processes that underlie the ability of the hydrosol to neutralize the toxic effects of imidacloprid, for example, antioxidant properties in hydrosol and activation of a mussel natural defense system.

If these goals are met, it should be possible to present a comprehensive picture of how green tea hydrosol can be used as a natural remedy for the poisonous effect of imidacloprid in order to accomplish safer and sustainable control of the zebra mussel population in aquatic environments.

1.4. Hypothesis

Imidacloprid's negative effects on zebra mussels may be lessened by green tea hydrosol's abundance of beneficial chemicals.

1.4.1. Green Tea Hydrosol's Rich Composition

One of the most famous of these hydrosols, green tea, is full of bioactive compounds such as polyphenols, catechins, and flavonoids. Antioxidant properties of these compounds have had them celebrated. These antioxidants may also play a role in mitigating the harmful effects of imidacloprid.

1.4.2. Oxidative Stress and Reactive Oxygen Species (ROS)

If zebra mussels are exposed to imidacloprid, it is expected it will produce reactive oxygen species (ROS) in their internal systems. Chemically reactive oxygen molecules that include oxygen can cause oxidative stress doing harm to cellular constituents, such as DNA, proteins and lipids. The zebra mussels' health and ability to function may be severely compromised by this oxidative damage.

1.4.3. Neutralizing ROS

Green tea hydrosol is hypothesized to neutralize these reactive oxygen species as the tea contains antioxidants, which capture free radicals. Doing so enables the antioxidants to decrease oxidative stress that may protect cellular structures and functions in the zebra mussels. Despite exposure to the toxic chemical, this protective action is critical to health of the mussels.

1.4.4. Enhancing Detoxification Processes

The bioactive compounds in green tea hydrosol might not stop by neutralizing ROS, and may also activate the mussels' own means of detoxification. This includes the enhancement of detoxifying enzymes and of other mechanisms such as clearing of harmful substances from their system. Such a boost would add to the alleviation of the toxic effects of imidacloprid.

Green tea hydrosol was therefore thought to reduce imidacloprid's harmful effects in zebra mussels. Due to the antioxidant content of green tea hydrosol, it may help to alleviate oxidative stress, and simultaneously work alongside mussels' natural detoxification processes to protect the mussels from chemical exposure. In order to test this hypothesis a set of experiments will be run to observe and measure these protective effects.

1.5. Significance of the Study

This study has relevance well beyond imidacloprid and zebra mussels. The most significant subjects covered are environmental protection, sustainable practices, and the potential for innovative natural solutions to the issues faced in keeping a planet healthy. But here's a detailed look at why this study matters.

1.5.1. Environmental Toxicology

This work also contributes to environmental toxicology by exploring a plant based, natural, solution for chemical pollution. Like most other pesticides, Imidacloprid is persistent and accumulates in the body, therefore threatening non target species and ecosystems. The study adds

to the knowledge base of safer alternatives to chemical solutions for these toxic effects by demonstrating the efficacy of green tea hydrosol in mitigating these toxic effects. It also indicates that natural materials may be utilized as detoxifying agents in the presence of harmfully contaminated substances that would otherwise require expensive artificial detoxifying agents having potentially harmful effects to the environment [23].

1.5.2. Aquatic Ecosystem Management

While most freshwater ecosystems are relatively protected from having existing predators, they are extremely vulnerable to pollution, invasive species and other anthropogenic pressures. The case of the zebra mussel as an invasive species is a paradigmatic example of the management challenges of ecosystems as a complex system. The findings of this study could have a significant impact on how to control invasive species and restore devastated habitats. If green tea hydrosol proves effective in protecting zebra mussels from the toxic effects of imidacloprid, it suggests a dual benefit: The ability to control invasive populations with little impact on other parts of the overall ecosystem. Such an approach creates more balanced, more healthy water environments [24].

1.5.3. Sustainable and Eco-Friendly Strategies

The development of sustainable and ecofriendly practices is one of the most pressing needs for environment management today. Pest and pollution control using traditional methods are often harsh chemicals that can eventually prove to be toxic for the environment. The alternative, however, is promising: natural solutions such as green tea hydrosol. Through the use of naturally occurring compounds that degrade more easily, with lower risk to non-target species, it becomes possible to devise strategies that are more in line with ecological principles and those of sustainability. Further studies could be made based on this as an insight into other plant-based substances that have detoxifying properties of this kind, representing more plant-based substance to choose from in order to protect our environment [25].

1.5.4. Mitigating Adverse Effects of Chemical Pollutants

Chemical pollutants have become ubiquitous in our world: from water quality to wildlife to human health. They are therefore crucial to find ways for mitigating their adverse effects. With a whole host of antioxidants and detoxifying compounds, green tea hydrosol represents a powerful, natural solution. Hence, it is very important to learn how it works to neutralize such toxins as imidacloprid and then use this information to create new solutions for pollution cleanup both in agricultural runoff and industrial waste situations. Cleaner waterways and healthier ecosystems can result and better public health outcomes [26].

1.5.5. Effective Management of Invasive Species

Zebra mussels are just one of many invasive species that disrupt native ecosystems and economy to the tune of millions of dollars in damages and control annually. Methods of managing these species have been traditionally performed using chemical treatments that can in turn cause harm to the same ecosystems need to conserve. Therefore, investigation of green tea hydrosol as a detoxifier gives a novel approach to this problem that may be less disruptive and more selective. Reducing toxicity of necessary chemical treatments allows us to manage invasive populations with less collateral damage made to native species and habitats. That is essential for the vitality of biodiversity and environmental resilience [27].

1.5.6. Preservation of Aquatic Biodiversity

Healthy ecosystems are based on biodiversity with its capacity to help buffer changes in the environment and also provide a diversity of functions. Contribution of the findings of this study to biodiversity conservation by providing means of protection of sensitive aquatic species from undesirable effects of pesticides. In addition, biodiversity in freshwater systems, where it is usually under threat from pollution, habitat destruction and climate change, is made more important by this. Promoting the use of natural detoxifiers will produce safer environments for many, and a wider array of aquatic organisms [28].

1.5.7. Contributions to Environmental Remediation

In addition to the context of zebra mussels and imidacloprid, this study carries other meanings for the field of environmental remediation. This also could help open up new ways to clean contaminated areas, by understanding how natural detoxifiers like green tea hydrosol interact with the pollutants. They also show that plant-based substances can be used to remediate polluted water bodies, soils and other ecosystems, and could be a more sustainable and less invasive method than conventional remediation techniques. In areas where the threat of chemical pollution to human and environmental health is high this could be especially useful [29].

1.5.8. Insights into Plant-Based Detoxifiers

In addition, the study contributes to the increasing body of knowledge regarding the potential of plant-based substances in environmental management. The research provides a basis for exploring other natural compounds with similar properties, by elucidation of the mechanisms through which green tea hydrosol mitigates toxicity. The potential for a more general application of plant-based detoxifiers in different environmental settings, such as in industrial waste treatment

and agriculture, suggests itself. The conclusions obtained could motivate innovation in both detoxification and pollution control approaches based on natural and sustainable solutions [30].

Collectively, this work holds the potential to make substantial advances towards both environmental toxicology and aquatic ecosystem management and management, as well as sustainability and environmental remediation. The application also highlights the future applications and innovations of environmental protection by showing the capacity of green tea hydrosol as a natural detoxifier. These findings suggest the way forward for more sustainable, less damaging ways to deal with chemical pollutants and protect the health and diversity of planet Earth's ecosystems.

1.6. Literature Review

1.6.1. Zebra Mussels (*D. polymorpha*) Biology and Ecology

Small, freshwater bivalve mollusks with distinctive, zebra like striped shells, zebra mussels (*D. polymorpha*) are easily identified by their shells. Native to freshwater lakes in southern Russia and Ukraine within the Black, Caspian and Azov Sea basins, these mussels are being put at very serious risk – by being sold to pubs and restaurants across the country. Zebra mussels natural history is an interesting one because of their reproductive ability and unique ecological traits. One feature that distinguishes zebra mussels as fish, and indeed as any other phylum, is the fact that they have a very high reproductive rate for biological traits. One female zebra mussel can produce more than million eggs per spawning season. These eggs become free swimming larvae called Veligers. Veligers are planktonic and may, therefore, be dispersed widely in water currents, resulting in rapid colonization of new habitats [31]. A key factor in the invasive success of zebra mussels is the ability to get around quickly [31]. Throughout the veliger phase of the life cycle, the veligers are able to swim and feed; once they settle, however, they undergo a metamorphosis to become juvenile mussels and begin secreting byssal threads. Zebra mussels adhere firmly to hard surfaces by means of these proteinaceous fibers, known as threads. They can settle on many different substrates, such as rocks, submerged wood, boat hulls, or in fact the shell of other mollusks. This ability to attach allows zebra mussels to form dense colonies which can become so thick as to blanket areas from natural habitats to man-made structures [32]. Zebra mussels are a filter feeder, filtering small particles – generally plankton – from the water column. The ecological impact of this feeding behavior is considerable. Zebra mussels are able to filter large volumes of water and, as a result, can dramatically increase water clarity via increased light penetration resulting in increased aquatic vegetation growth. But this also means they are capable of removing large amounts of phytoplankton a primary food source for many native aquatic species. This decrease in

phytoplankton can affect food webs and has repercussions for native filter feeding organisms eat some species of fish and invertebrates [33].

1.6.2. Invasive Nature and Ecological Impact

The introduction of zebra mussels to North America exemplifies the ecological devastation that invasive species can cause to economies and ecosystems. First appeared about a decade ago in North America after being discovered in the late 1980s and thought to have been brought over in ballast water discharged from ships originating from European waters. Their initial discovery on Lake St. Clair between Lake Huron and Lake Erie began the rapid spread of them throughout the Great Lakes and other USA and Canadian freshwater systems [34]. A number of factors are attributed to the invasive success of zebra mussels. They have a high reproductive output and a high growth rate, meaning they can quickly establish and expand populations. Like most invasive species, zebra mussels have a strong ability to outcompete native species for resources. As filter feeders they filter large volumes of water to feed on plankton, which can starve out native species by starving them out. The use of zebra mussels is made more competitive due to the fact that they can attach to nearly any substrate, and often smother native mussel beds and other bottom dwelling organisms. [35]. An extensive ecological impact has been left by zebra mussels. Once zebra mussels become established in ecosystems, they can change water chemistry and clarity, affect nutrient cycling, and disturb local food webs. Their filtration activity raises water clarity, but it may promote overgrowth of benthic algae and submersed aquatic vegetation. This decrease in primary production could reverberate throughout the ecosystem, influencing everything from invertebrates to fish populations [36]. Economically zebra mussels are just as problematic. Biofouling, which is characterized by the accumulation of organisms on submerged surfaces, is notorious for being caused by them. Zebra mussels clog water intake pumps, foul boat hulls, and damage to industrial and municipal water systems. The costs to manage and mitigate these impacts are large. Zebra mussel infestations increase maintenance costs and operational challenges of industries that depend upon water intake systems including power plants and municipal water facilities. Zebra mussels have cost the power industry billions of dollars in maintenance and damage control, according to U.S. Fish and Wildlife Service estimates [34].

1.6.3. Control Methods

Controlling zebra mussel populations is a complicated and always growing concern that needs to be addressed through a variety of means. They used different methods which each have their advantages and disadvantages. Broadly these methods can be classified as mechanical, chemical, and biological controls.

Mechanical Removal

The physical techniques use some methods to remove zebra mussels from infested areas. These methods may cover scrubbing, high pressure washing and manual scraping. Though it has proven effective for cleaning certain surfaces, mechanical removal is labor intensive, and ultimately only a short-term solution, as mussel populations can quickly recover. Mechanical methods are not practical for large scale infestations, especially where access to large infestations such as in natural water bodies are restricted [37].

Chemical Treatments

Use of molluscicides to kill zebra mussels is known as chemical control. Such a chemical as imidacloprid is a neonicotinoid insecticide that is used in this way. It causes them to collapse their nervous system, paralyze and suffocate them. However, chemical treatments are plagued by serious environmental concerns. Molluscicides can be toxic to non-target species such as beneficial invertebrates and fish and amphibians. They must also be managed to minimize risks to human health and water quality issues. Additionally, chemicals such as imidacloprid may remain in the environment for long periods, creating long term ecological effects [38].

Biological Control

In addition, because traditional chemical control methods can be byproducts of environmental degradation themselves, more environmentally friendly biological control methods are being explored. These introductions of natural predators, parasites, or pathogens specific to zebra mussels represents other alternative approaches. An example is the employment of some species of fish that feed on zebra mussels; however, this approach is hobbled by the availability and efficiency of those predators. Another promising alternative is to utilize bacterial pathogens, or viruses, that infect and kill zebra mussels. But biological control methods must be used with regard to ecological balance and the possibility of undesirable side effects. The introduction of new species or pathogens into an ecosystem introduces the possibility of unexpected consequences to non-target species or ecosystem health [39].

Integrated Pest Management (IPM)

A growing popular approach is Integrated Pest Management (IPM) that incorporates using numerous control techniques to affect the managing of zebra mussel populations sustainably. IPM is generally concerned with the use of environmentally sound practices and the minimum use of chemical treatments. It often involves monitoring zebra mussel populations communicating, mechanical and biological controls, and chemicals only as a last resort. The objective is to limit zebra mussel damage while maintaining ecosystem integrity [40].

Finally, zebra mussels have shown to be invasive; rapidly colonizing new habitats which are having significant ecological and economic impact to native ecosystems. Management has to achieve balance of mechanical, chemical, and biological methods, each having its own benefits and limitations. Further research and innovation are needed to improve strategies for the control of zebra mussel populations, and to reduce the effect of zebra mussels on freshwater ecosystems.

1.7. Imidacloprid

1.7.1. Chemical Properties and Usage

A member of the neonicotinoid class of insecticide, imidacloprid is part of an increasingly popular group of insecticides that display high efficacy and a marked degree of selectivity towards insects. Chemically, imidacloprid bears a chloronicotinyl group, which is of particular importance with regard to mode of action. The structural feature of this enables imidacloprid to bind with high affinity to the nicotinic acetylcholine receptors (nAChRs) on in the nervous system of the insects, paralyzing and killing it [41]. The differences in structure and function between insect and mammalian nAChRs are thought to explain the selective toxicity of imidacloprid. In insects, the receptors are more sensitive to the neonicotinoids than in mammals, so they are less toxic to them human and domestic animals. Because of this selectivity, imidacloprid is among the most commonly used insecticide worldwide and its use extends to a number of agricultural use including the protection of crops including cotton, rice, cereals, and vegetables from a wide variety of pests including aphids, beetles, and whiteflies [42]. In addition, in non-agricultural settings such as for the control of termites, fleas and other household pests Imidacloprid is used. Because of its versatility and effectiveness, it is used in many pest control products sprays, granules, seed treatments, pet collars, etc. While common in use, scientists and environmentalists have been concerned about the persistence of imidacloprid in the environment [43].

1.7.2. Mechanism of Action

The principal mode of action of imidacloprid as an insecticide is through its interaction with intestinal and central nervous system nicotinic acetylcholine receptors (nAChRs) of insects. These receptors normally bind to neurotransmitter acetylcholine triggering a cascade of neural impulses necessary for a muscle contraction and other important events in physiology. Imidacloprid is an acetylcholine mimic, but it binds to these nAChRs and they do not degrade nearly as fast. Continuous stimulation of the nervous system that results from this persistent binding prevents normal synaptic transmission and eventually leads to neuronal exhaustion and paralysis, and death of the insect [44]. This mechanism is very effective against target pests but ultimately poses risk to

non-target organisms because imidacloprid is highly persistent in the environment. Applied once, once imidacloprid is applied it stays in soil and water for prolonged periods of time due to things like make-up of the soil, temperature, microbiological activity. In soil it can have a half-life measured in weeks to months and in aquatic environments, under some conditions, it persists for even longer [45]. Imidacloprid accumulates in soil, which results in persistence that can expose non target species beneficial insects, crustaceans, fish, and amphibians to sublethal doses for long periods. Prolonged exposure to imidacloprid may produce several sub-lethal effects, including reduced reproduction, growth and altered behavior, and these effects can have shambles on ecosystems [46].

1.7.3. Environmental Impact and Toxicity

It has been widely used and persisted in the environment, making the imidacloprid environmental impact the subject of extensive research in its entirety. Imidacloprid has been found in a wide range of environmental matrices, e.g. water bodies, sediments and soils, and issues related to possible effects on non-target species and ecosystems were raised.

Impact on Aquatic Ecosystems

The high toxicity of imidacloprid to aquatic invertebrates makes its occurrence in aquatic environments of growing concern. Exposure to imidacloprid has been shown to lead to significant mortality and sublethal effects to crustaceans, mollusks and insects. For example, showed that imidacloprid exposure reduced the survival of, and impaired reproduction and development in, aquatic invertebrates. Disruption of freshwater aquatic food webs and decrease the freshwater aquatic biodiversity may induce the effects of these [47].

Effects on Non-Target Terrestrial Invertebrates

Apart from aquatic ecosystems, terrestrial invertebrates, especially pollinators, such as bees, are also at risk from imidacloprid. Sublethal effects of neonicotinoids, including imidacloprid, on foraging behavior, navigation and colony health have been implicated in pollinator population decline. Imidacloprid exposure, at even low levels can cause bees to be less able to forage for food, to navigate their way back to their hives, and to reproduce, all behaviors linked to colony collapse disorder [48].

Soil Health and Microbial Communities

Imidacloprid can also disrupt the microbial community, which is essential for nutrient cycling and soil structure preservation. Imidacloprid present in the soil alters composition and function of microbial communities with detrimental effect for microbial ecosystem services.

Human Health Risks

Imidacloprid, like most insecticides, is selectively toxic, meaning that although it is not toxic to humans under normal conditions, it can produce serious health effects to agricultural workers who are regularly exposed to this pesticide. Chronic exposure to imidacloprid can supposedly be tied to neurological symptoms, reproduction issues and developmental effects, according to studies. Regulatory agencies deem the levels of imidacloprid found in food to be safe however, research into the long-term effects of low-level exposure is ongoing [49].

Regulatory and Mitigation Measures

As the evidences of the contributions of imidacloprid to the health and the environment pile up, several countries have imposed regulatory restrictions to its use. For example, the European Union has banned the use of imidacloprid and other neonicotinoids, which pollinate and aquatic ecosystem protection. The Environmental Protection Agency (EPA) in the United States is still assessing risk posed by neonicotinoids and has taken steps to reduce exposure in sensitive places.

Finally, despite its effectiveness as a pest management tool, the environmental persistence and possibility of toxicity to non-target species of imidacloprid is problematic. Sustainable and safer alternatives are clearly needed and research into natural detoxifiers and integrated pest management strategies continues to be critical to offsetting the detrimental effects of imidacloprid on environmental systems and human health.

1.8. Green Tea (*C. sinensis*) Hydrosol

1.8.1. Composition and Bioactive Compounds

The leaves of *C. sinensis* which yields green tea contain a variety of bioactive compounds such as polyphenols, catechins and flavonoids. These are mainly responsible for green tea's well known health benefits and potential therapeutic properties. Green tea hydrosol is made during the process of steam distillation used to extract essential oils from green tea leaves. This hydrosol carries many of the valuable bioactive compounds originally contained in the plant material [50]. The key constituents that make up green tea are catechins, which are a type of polyphenol including the epigallocatechin gallate, epicatechin gallate, epicatechin and epigallocatechin. Catechins in this form have potent antioxidant properties. In particular, EGCG is one of the most abundant and biologically active catechins present in green tea and likely plays an important part in most of green tea's health promoting effects. Along with catechins, green tea also provides the additional polyphenols like flavonoids and tannins that are able to add oxidative capacity. Together, these compounds have the synergy to make them much more effective as anti-oxidant and bio health

contributors as a whole. Although a byproduct, green tea hydrosol makes a substantial uptake of these bioactive compounds and serves as a rich resource for several therapeutic applications [51].

1.8.2. Antioxidant and Detoxifying Properties

Green tea polyphenols are known for their antioxidant properties and are the basis for the protective effects of green tea against a number of different diseases or conditions associated with oxidative stress. Oxidative stress is an imbalance between a person's reactive oxygen species (ROS) and the detoxification and repair processes the body uses, and is measured in terms of 'ROS oxidant excess'. Damages to DNA, proteins, and lipids by excessive ROS is common in various pathological conditions. Studies have shown that green tea extracts increase the activity of endogenous antioxidant enzymes for example superoxide dismutase (SOD) and catalase. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radicals reactive oxygen radicals into molecular oxygen and hydrogen peroxide; while catalase cleaves hydrogen peroxide to water and molecular oxygen. Green tea polyphenols increase the activity of these enzymes in order to mitigate oxidative stress, and protect cells from damage [52]. Additionally, it is known that green tea polyphenols function to modulate detoxification pathways. Researchers have shown that they can induce the expression of phase II detoxifying enzymes for example, glutathione S-transferases, GSTs. GSTs are critical to the detoxification process in which a toxic substance is conjugated to glutathione, such that the resulting conjugate is more water soluble and thus more easily eliminated by the body. The potential for green tea hydrosol as a natural detoxifying agent appears in this study to lie in the dual action of direct antioxidant activity and the ability to enhance detoxification pathways, and thereby facilitate detoxification.

1.8.3. Applications in Environmental Toxicology

Given that application of green tea hydrosol in environmental toxicology is still in its newly emerging phase, and with its ability to counter the adverse effect associated with environmental toxicants, this work is highly promising in environmental toxicology. Potential use of green tea hydrosol as a natural detoxifier has been examined in several settings, showing that it lowers oxidative stress, and protects against chemical induced damage in several organisms. However, the potential of green tea hydrosol as a safe and natural detoxifier is not limited to heavy metals and pesticides. Because green tea hydrosol has a rich mixture of bioactive compounds, it could also work to detoxify other environmental poisons, organic solvents or industrial chemicals. Green tea hydrosol reduces oxidative stress and promotes detoxification pathways and protects organisms from the deleterious effects of environmental contaminants [53]. Moreover, green tea hydrosol can be used for environmental toxicology in which the use of green tea in detoxifiers have green, safe,

and efficient origin. Green tea hydrosol is a natural product and is easily biodegradable and less likely causing secondary pollution, so it has great potential for environmental remediation work. Green tea hydrosol is very richly laden with bioactive compounds and should be regarded as a worthwhile natural detoxifying agent. By reducing environmental pollutants, it can help mitigate the harmful effects of those pollutants, and be a sustainable way to protect ecosystems and improve environmental health for years and decades and centuries to come.

1.9. Interaction Between Pollutants and Natural Detoxifiers

1.9.1. Case Studies and Previous Research

An emerging field with significant promise is the interaction between environmental pollutants and natural detoxifiers as a means to mitigate the adverse effects of many other contaminants. Increasingly, research has shown that plant substances could neutralize toxins or even remove them and reduce, or potentially reverse, environmental damage. Notable case studies and research are described in this section, which underpin these interactions. One comprehensive study by C. Xu [54] Various plant extract effects against heavy metal toxicity in aquatic organisms were investigated. Pervasive pollutants, such as lead, cadmium and mercury, are heavy metals that can present serious hazards to aquatic life and ecosystems. In this research, Xu and colleagues [54] sifted through several plant extracts that have a significant number of antioxidants like green tea, turmeric and garlic. Findings were compelling, antioxidant rich extracts reduced the oxidative stress markers and increased the survival rates of aquatic organisms exposed with heavy metals. As the study showed, plant-based antioxidants have the potential to reverse heavy metal induced oxidative damage. Another pertinent study by E.S. Kim [55] the potential use of plant derived antioxidants to increase the resilience of aquatic ecosystems to pollutant induced stress was explored. Kim [55] studied the use of green tea polyphenols to reduce the effects of pesticide exposure in fish. Like heavy metals, pesticides cause significant oxidative stress that results in cellular damage and reduced organism health. Fish treated with green tea polyphenols had lower levels of oxidative stress and higher survival rates compared to untreated controls, according to the study. These results indicate that green tea polyphenols can 'help boost' the existing defenses of aquatic organisms, allowing them to cope better with environmental pollutants [56]. The requirements of plant-based detoxifiers also contributed to the understanding. In order to study the effects of green tea polyphenols in rats with pesticide induced oxidative damage, D. Komes and colleagues [56] examined rats exposed to pesticide. According to their results, green tea polyphenols would increase their antioxidant enzyme activities, shall lower their lipid peroxidation, and enhance their overall cellular condition. This study showed the green tea polyphenols potential as effective detoxifiers as varying species and environmental contexts. Collectively, these case studies illustrate

the great potential of plant-based substances, such as green tea hydrosol, to more effectively neutralize environmental pollutants. These natural compounds are able to reduce oxidative stress and increase resilience in aquatic ecosystems is a path of development for sustainable environmental management.

1.9.2. Mechanisms of Detoxification

The mechanisms by which natural substances like green tea hydrosol detoxify pollutants need to be understood in order to devise effective environmental remediation strategies. Green Tea hydrosol may detoxify by several interrelated pathways; these include its abundant antioxidants and bioactive compounds.

Neutralizing Reactive Oxygen Species (ROS)

Neutralization of ROS generated from exposure to pollutants is one of the major detoxification mechanisms. ROS are oxygen related free radicals, superoxide radicals, hydrogen peroxide and hydroxyl radicals. These molecules can inflict important oxidative stress to a lipid, protein, or DNA components of cells. The antioxidant ability of green tea polyphenols, in particular catechins such as EGCG, is strong due to its ability to scavenge ROS. Green tea hydrosol neutralizes these reactive species and thus helps protect cellular structures maintaining cellular function [57].

Enhancing Antioxidant Enzyme Activity

In addition, these green tea polyphenols can improve the activity of endogenous antioxidant enzymes. Some enzymes relieve oxidative stress including superoxide dismutase (SOD), catalase, and glutathione peroxidase. SOD turns superoxide radicals into hydrogen peroxide, which catalase and glutathione peroxidase split down into water and oxygen. A number of studies have confirmed that green tea extracts can increase the activity of these enzymes, which increases the organism's natural antioxidant defenses [58].

Modulating Detoxification Pathways

Another important mechanism has to do with modulation of detoxification pathways. Green tea polyphenols have been reported to cause expression of phase II detoxifying enzymes including glutathione S transferases (GSTs). GSTs act as catalyzers to conjugate toxic substances to the molecule glutathione, creating a more water-soluble compound which the body can more easily eject. This is an essential part of detoxing of heavy metals and pesticides among other environmental toxins [58].

Activating the Nrf2 Signaling Pathway

The nuclear factor erythroid 2 related factor 2 (Nrf2) signaling pathway is a key regulator of cellular antioxidant responses. The expression of a great number of genes implicated in antioxidant defense and detoxification is under Nrf2 control. The Nrf2 pathway induced by green tea polyphenols, specifically EGCG, has been demonstrated. Nrf2 activation results in the increase of antioxidant and detoxification enzymes expression in the organism, and serves to promote the organism's defense against, and detoxification of, toxicants. Maintaining cellular redox balance and protecting against oxidative damage is, however, critical for this pathway [59].

Improving DNA Repair Mechanisms

Green tea polyphenols in addition to neutralizing ROS and enhancing antioxidant defenses, can also enhance DNA repair mechanisms. Various forms of DNA damage e.g. single strand breaks and base modifications are caused by oxidative stress. For keeping genomic integrity and avoiding mutation, it is indispensable to have an effective DNA repair. Green tea polyphenols can enhance the activity of DNA repair enzymes contributing to the repair of oxidative DNA damage, and the reduction of the risk of mutagenesis [60].

This work explores the detoxifying properties of green tea hydrosol and its interaction with pollutants such as imidacloprid to help develop sustainable approaches for environmental toxicity management. Knowledge of these interactions can facilitate the development of broader environmental remediation and conservation applications of natural detoxifiers. From the aforementioned the potential value of green tea hydrosol that can be used as an effective tool to prevent harmful effects of environmental pollutants caused by various mechanisms uniting its detoxification capabilities was recognized [61].

2. MATERIAL AND METHOD

2.1. Objective and Overview of the Study

In this study, the detoxifying potential of green tea (*C. sinensis*) hydrosol was examined against the toxic effects of imidacloprid on zebra mussels (*D. polymorpha*). Part of the work involved a detailed investigation of the biochemical and physiological responses of the mussels to both the insecticide and the hydrosol. The experimental design was structured around several key steps:

- Collection and maintenance of zebra mussels.
- Preparation of imidacloprid solutions.
- Preparation of green tea hydrosol.
- Assessment of acute and chronic toxicity.
- Biochemical and physiological analyses.

2.2. Preparation of Green Tea Hydrosol

The plant from which the green tea used in this process comes from is *Camellia sinensis*. The hydrosol of green tea was supplied from a commercial company (Altınterim Natural Health Company, Elazığ, Türkiye). The hydrosols were kept in sterile dark colored bottles (500 ml) under refrigerated conditions until use [62, 63].

2.3. Experimental Design and Methodology for Zebra Mussel Toxicity Study

Specific experimental design and methodology to determine toxicity factors of imidacloprid and possible detoxification by green tea hydrosol on the zebra mussels (*D. polymorpha*). Imidacloprid was used to expose zebra mussels to several intermediate concentrations and green tea hydrosol was added at different rates.

2.4. Test Organism

2.4.1. Species

The test organism in the present study was the zebra mussel (*D. polymorpha*), a widely used bioindicator organism in environmental toxicity studies. Because of their filter feeding nature and high sensitivity to pollutants, zebra mussels were ideal candidates for assessment of the impact of chemical contaminants in aquatic ecosystems.

2.4.2. Mussels Source

For this experiment, the mussels were collected from the Keban Dam Lake (Pertek Hunting Area) in Türkiye, where zebra mussels are part of the normal natural habitat.

2.5. Adaptation to Laboratory Conditions

One week before beginning experiments, the zebra mussels were acclimated. This kept the mussels from being able to rapidly adapt to lab conditions and they were in good health for the experiment. Figure 2.1 shows zebra mussels in lab aquarium. During acclimatization:

- **Water:** Mussels were kept in dechlorinated water tanks in which there were no harmful amounts of chlorine to disrupt their physiology.
- **Temperature:** Zebra mussels thrived in the water at an appropriate temperature range, usually between 18 – 22°C.
- **Lighting:** Natural daily light cycles were maintained to simulate natural conditions and mussels were maintained such that normal behavior and physiological processes were kept.
- **Acclimatization Duration:** The mussels condensed to the new environment required a week, to ensure stable consistent conditions throughout the experiment.



Figure 2.1. Zebra Mussels in Aquarium

2.6. Determination of LC50

• The LC50 (Lethal Concentration 50%) of the common pesticide imidacloprid was taken from the literature rather than going through the work of determining the LC50 through preliminary testing [64]. The 0.01 µg L⁻¹ concentration was the acute toxicity concentration for freshwater

invertebrates including zebra mussel and the 0.385 $\mu\text{g L}^{-1}$ was the chronic toxicity concentration [58, 65].

2.7. Experimental Setup

The experimental design consisted of control versus exposure groups with different imidacloprid concentrations and different concentrations of green tea hydrosol treatments. These mussels were then placed in polycarbonate tanks, each held in 1 liters of test water and sufficient ventilation. There were 8 mussels per group. Groupings and Exposure Conditions

2.7.1. Control Groups (CA, C1, C2, C3, C4)

They were not exposed to imidacloprid. Instead, I used the mussels as controls in the study to see how their health was affected by other levels of green tea hydrosol exposure. Figure 2.2 and Table 2.1 shows experimental setup and exposure matrix respectively.

- **Control Group CA:** Mussels were kept in clean water for the entire duration of the study.
- **Control Group C1:** Mussels received 10 ml/L of green tea hydrosol for 192 hours without imidacloprid exposure.
- **Control Group C2:** Mussels received 20 ml/L of green tea hydrosol for 192 hours without imidacloprid exposure.
- **Control Group C3:** Mussels received 40 ml/L of green tea hydrosol for 192 hours without imidacloprid exposure.
- **Control Group C4:** Mussels received 80 ml/L of green tea hydrosol for 192 hours without imidacloprid exposure.

2.7.2. Exposure Groups (A1, A2, A3, A4 & B1, B2, B3, B4)

Different concentrations of imidacloprid, in presence of different rates of addition of green tea hydrosol as described below, were exposed to these groups.

- **Low Concentration Group A1:** Mussels were exposed to a low concentration of imidacloprid (1 $\mu\text{g/L}$) for 96 hours, followed by the addition of 10 ml/L of green tea hydrosol for another 96 hours.
- **Low Concentration Group B1:** Mussels were exposed to a low concentration of imidacloprid (1 $\mu\text{g/L}$) and simultaneously received 10 ml/L of green tea hydrosol for 192 hours.

- **Medium Concentration Group A2:** Mussels were exposed to a medium concentration of imidacloprid (10 µg/L) for 96 hours, followed by the addition of 20 ml/L of green tea hydrosol for another 96 hours.
- **Medium Concentration Group B2:** Mussels were exposed to a medium concentration of imidacloprid (10 µg/L) and simultaneously received 20 ml/L of green tea hydrosol for 192 hours.
- **High Concentration Group A3:** Mussels were exposed to a high concentration of imidacloprid (100 µg/L) for 96 hours, followed by the addition of 40 ml/L of green tea hydrosol for another 96 hours.
- **High Concentration Group B3:** Mussels were exposed to a high concentration of imidacloprid (100 µg/L) and simultaneously received 40 ml/L of green tea hydrosol for 192 hours.
- **Very High Concentration Group A4:** Mussels were exposed to a very high concentration of imidacloprid (1000 µg/L) for 96 hours, followed by the addition of 80 ml/L of green tea hydrosol for another 96 hours.
- **Very High Concentration Group B4:** Mussels were exposed to a very high concentration of imidacloprid (1000 µg/L) and simultaneously received 80 ml/L of green tea hydrosol for 192 hours.



Figure 2.2. Experimental Setup

Table 2.1. Exposure Matrix (Hydrosol & Imidacloprid)

Group	Hyrdosol Dose (mL/L)	Imidacloprid Dose (µg)	Duration (Hours)	Description
CA	0	0	192	Control group with mussels in simple water.
C1	10	0	192	Mussels in 1L water + 10mL hydrosol for 192 hours.

C2	20	0	192	Mussels in 1L water + 20mL hydrosol for 192 hours.
C3	40	0	192	Mussels in 1L water + 40mL hydrosol for 192 hours.
C4	80	0	192	Mussels in 1L water + 80mL hydrosol for 192 hours.
A1	10	1	96+96	Mussels in 1L water + 10mL hydrosol for 96 hours, then 1µg imidacloprid added, continued for 96 hours.
A2	20	10	96+96	Mussels in 1L water + 20mL hydrosol for 96 hours, then 10µg imidacloprid added, continued for 96 hours.
A3	40	100	96+96	Mussels in 1L water + 40mL hydrosol for 96 hours, then 100µg imidacloprid added, continued for 96 hours.
A4	80	1000	96+96	Mussels in 1L water + 80mL hydrosol for 96 hours, then 1000µg imidacloprid added, continued for 96 hours.
B1	10	1	192	Mussels in 1L water + 10mL hydrosol + 1µg imidacloprid for 192 hours.
B2	20	10	192	Mussels in 1L water + 20mL hydrosol + 10µg imidacloprid for 192 hours.
B3	40	100	192	Mussels in 1L water + 40mL hydrosol + 100µg imidacloprid for 192 hours.
B4	80	1000	192	Mussels in 1L water + 80mL hydrosol + 1000µg imidacloprid for 192 hours.

2.8. Experimental Duration and Monitoring

- **Duration:** The experiment lasted for 192 hours, and Groups Low to Very High.
- **Water Quality Monitoring:** During the experiment, water quality parameters (temperature, pH and dissolved oxygen) were continuously monitored and their values kept at suitable levels to maintain mussel health and stability.

2.9. Biochemical Analysis

The mussels were euthanized at the end of the 8-day exposure period. Detoxification responses were evaluated by biochemical analyses. Potential biomarkers of interest included:

- **Enzyme Activity:** This will allow the analysis of key detoxification pathway enzymes, such as antioxidant enzymes (e.g., catalase, superoxide dismutase).
- **Oxidative Stress Markers:** The extent of damage resulting from imidacloprid exposure was measured by the oxidative stress markers (e.g. Malondialdehyde).

At the completion of this experiment, the study was intended to give insight into the possible protective effect of green tea hydrosol against imidacloprid toxicity in aquatic invertebrates.

2.10. Observations and Measurements

It has monitored several toxicity indicators with regards to the effect of imidacloprid and green tea hydrosol to zebra mussels (*D. polymorpha*). The following findings were recorded:

2.10.1. Behavioral Changes

Throughout the experiment, the mussels exhibited minimal behavioral changes across all groups.

Valve Movement

- Mussels in the control groups (CA, C1, C2, C3, and C4) maintained regular valve opening and closing behavior.
- Mussels in treated groups (A1, A2, A3, A4, B1, B2, B3, and B4) showed slightly reduced valve movement during the first 48 hours of exposure to imidacloprid. However, this behavior normalized as the experiment progressed.

Response to External Stimuli

- All groups retained their ability to respond to external stimuli such as water flow or gentle tapping.

Egg Deposition

- Eggs were observed on the surface of a few mussels in groups treated with green tea hydrosol (C3, C4, and A4). This suggests potential stimulation of reproductive activity, which may be attributed to the antioxidant properties of the hydrosol. Figure 2.3 shows egg deposition on zebra mussel.



Figure 2.3. Zebra Mussels with Egg Deposition

2.10.2. Mortality

- The overall mortality rate was extremely low during the experiment:

Control Group (CA)

- One mortality was recorded at the end of the experiment.

Treated Group (A1)

- One mortality was observed in this group.
- All other groups had no recorded mortalities.

The minimal mortality across groups suggests that imidacloprid, at the tested concentrations, and the addition of green tea hydrosol did not impact survival rates during the experiment.

2.10.3. Physical Abnormalities

Shell Condition:

- No significant shell damage or discoloration was observed in any group.
- The mussels' shells remained smooth and intact throughout the experiment.

Soft Tissue Condition:

- No signs of soft tissue discoloration, swelling, or lesions were noted in any group.

Overall Appearance:

- Mussels in hydrosol-treated groups appeared healthy, with no visible signs of stress or abnormalities. Table 2.2 shows observed abnormalities in zebra mussels.

Table 2.2. Observed Physical Abnormalities

Group	Hydrdosol Dose (mL/L)	Imidacloprid Dose (µg)	Duration (Hours)	Mortality	Abnormalities	Eggs observed
CA	0	0	192	1/8	None	None
C1	10	0	192	0/8	None	None
C2	20	0	192	0/8	None	None
C3	40	0	192	0/8	None	Few
C4	80	0	192	0/8	None	Few
A1	10	1	96+96	1/8	None	Few
A2	20	10	96+96	0/8	None	None
A3	40	100	96+96	0/8	None	None
A4	80	1000	96+96	0/8	None	Few
B1	10	1	192	0/8	None	None
B2	20	10	192	0/8	None	None
B3	40	100	192	0/8	None	None
B4	80	1000	192	0/8	None	None

2.10.4. Observational Highlights

Egg Deposition

- The presence of eggs on the mussels' surface in hydrosol-treated groups indicates a potential stimulatory effect of green tea hydrosol on reproductive activity.

Mortality

- The negligible mortality suggests that both imidacloprid at tested doses and hydrosol did not induce lethal effects under the experimental conditions.

Physical Health

- The absence of abnormalities across all groups emphasizes the protective and non-toxic effects of green tea hydrosol.

These observations also indicate that imidacloprid is least toxic at low concentrations and that green tea hydrosol has potential beneficial role in maintaining mussel health.

2.11. Biochemical Responses: ELISA Analysis of Mussel Tissue

2.11.1. Introduction

On completion of the exposure period (192 hours) biochemical responses in mussels were measured for the oxidative stress and detoxification biomarkers [66]. The following parameters were measured using enzyme-linked immunosorbent assay (ELISA):

- **Enzyme Activities:** As indicators of antioxidative responses were superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX).
- **Oxidative Stress Markers:** Lipid peroxidation was studied using malondialdehyde (MDA). Table 2.3 shows list of selected biomarkers.

The function of antioxidant enzymes Catalase (CAT), Glutathione Peroxidase (GPX), Malondialdehyde (MDA) and Superoxide Dismutase (SOD), and oxidative stress indicators were evaluated by ELISA assays. In order to find meaningful differences between experimental groups, statistical analysis including ANOVA and post-hoc Tukey HSD tests were performed. The tabulated data are accompanied by graphical representations to help improve the understanding of the results.

Table 2.3. Purpose of Selected Biomarkers

Biomarker	Purpose
Catalase (CAT)	To measure antioxidant activity related to hydrogen peroxide degradation.
Glutathione Peroxidase (GPX)	To measure antioxidant activity related to the reduction of lipid peroxides.
Malondialdehyde (MDA)	To quantify lipid peroxidation as an indicator of oxidative stress.
Superoxide Dismutase (SOD)	To measure the antioxidant defense against superoxide radicals.

2.11.2. Tissue Collection

- At the end of the experiment, mussels were sacrificed, and their soft tissues were carefully dissected. Figure 2.4 shows tissue collection procedure.



Figure 2.4. Zebra Mussels Soft Tissue Collection

- Approximately 0.5 g of tissue was taken from each mussel and homogenized in 1 mL of phosphate-buffered saline (PBS, pH 7.4) and beads using a homogenizer. Figure 2.5, Figure 2.6 and Figure 2.7 shows set-up of experimental vials and homogenizing procedure respectively.



Figure 2.5. Experimental Sample Vials

- The homogenates were centrifuged at 3000 rpm for 20 minutes at 4°C. The supernatant was collected and stored at -20°C until analysis. Figure 2.8 shows procedure of centrifugation.



Figure 2.6. Homogenizer



Figure 2.7. Samples in Tubes During the Homogenization Process for Biomarker Analysis



Figure 2.8. Centrifuge Loaded with Samples for Separation of Biomarker Extracts

2.11.3. ELISA Procedure

The analyses for SOD, CAT, GPx, and MDA were performed using commercially available ELISA kits. The protocol followed the double-antibody sandwich method as outlined below:

Plate Preparation:

- The 96-well microplate pre-coated with the specific monoclonal antibody for each parameter was brought to room temperature before use.
- Standards and samples were prepared according to the kit instructions.

Standard Preparation:

- Serial dilutions of the provided standard were prepared to generate a standard curve for each assay (e.g., 4 ng/mL to 64 ng/mL for SOD).

Sample Loading:

- 50 μ L of each standard and 50 μ L of tissue supernatant samples were added to designated wells.
- For each test, duplicate wells were used for each standard, blank, and sample to ensure accuracy.

Antibody Incubation:

- 10 μ L of biotin-labeled antibody and 50 μ L of Streptavidin-HRP were added to the test wells. The plate was gently tapped to mix the contents.
- The plate was sealed and incubated at 37°C for 60 minutes.

Washing:

- After incubation, the plate was washed manually or using an automated washer with 0.35 mL of wash buffer per well. This step was repeated five times.

Color Development:

- 50 μ L of Chromogen Solution A and 50 μ L of Chromogen Solution B were added to each well.
- The plate was incubated at 37°C for 10 minutes in the dark.

Reaction Termination:

- 50 μ L of Stop Solution was added to each well, turning the color from blue to yellow.

Optical Density Measurement:

- The absorbance was measured at 450 nm using a microplate reader within 15 minutes of adding the stop solution. Figure 2.9 shows ELISA procedure.



Figure 2.9. Procedure of ELISA Assay

2.11.4. Data Analysis

After performing ELISA, the absorbance values of each sample were measured using an ELISA microplate reader at a specific wavelength (450 nm). The optical density (OD) values were recorded, and the mean and standard deviation (SD) for each group were calculated. These values represent the concentration of the biochemical markers in the mussel tissue samples, reflecting the oxidative stress and antioxidant responses. Figure 2.10 and Figure 2.11 shows data analysis of ELISA plates by ELISA microplate reader.

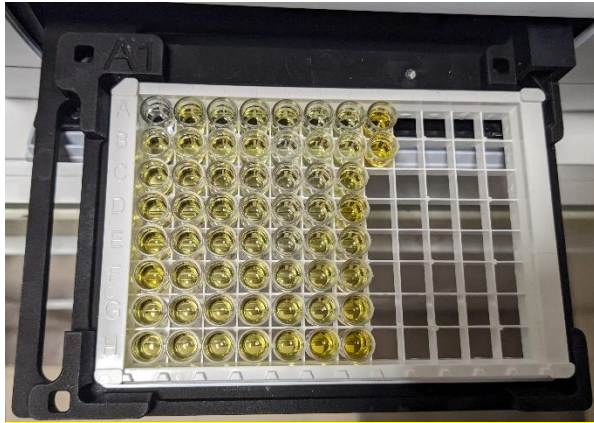


Figure 2.10. Enzyme-Linked Immunosorbent Assay

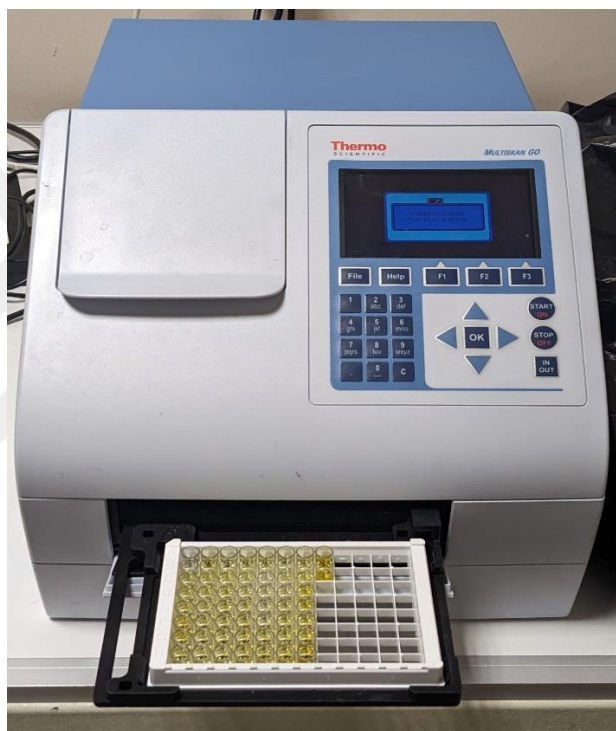


Figure 2.11. Reading ELISA results with a microplate reader

3. RESULTS

3.1. Catalase (CAT) Activity

Catalase activity, an indicator of antioxidant response, was assessed in all experimental groups. The findings are encapsulated and illustrated graphically in Figure 3.1.

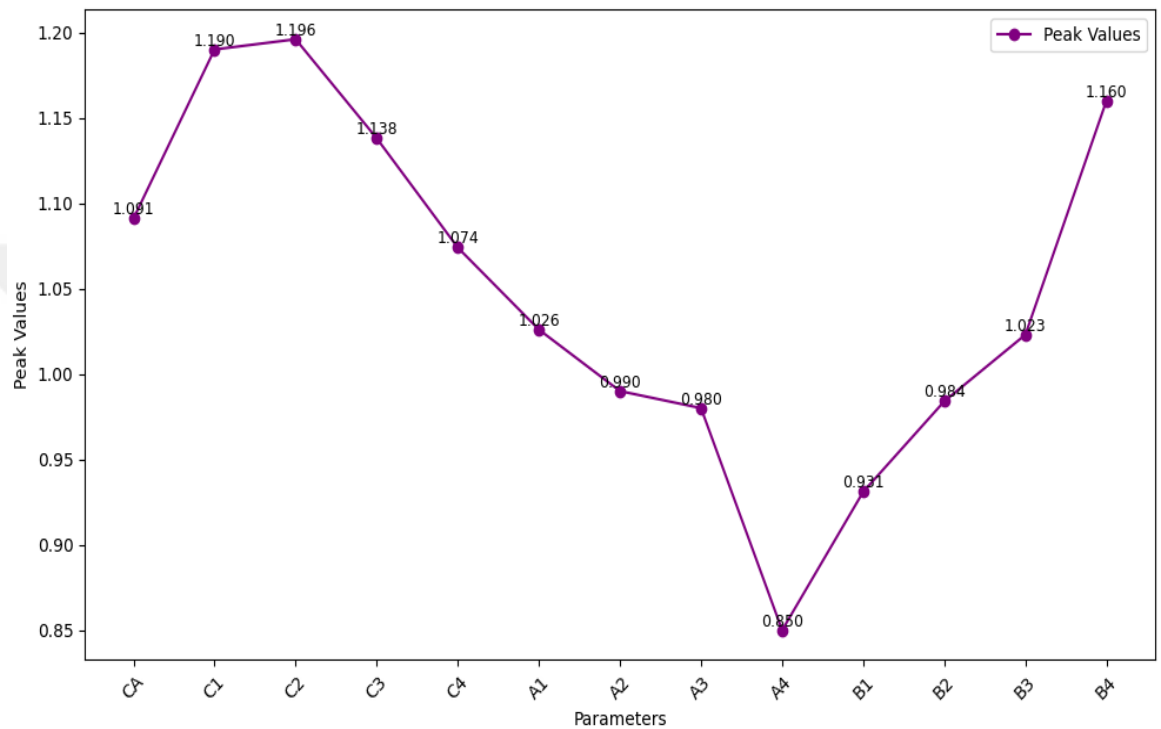


Figure 3.1. Catalase (CAT) Activity ELISA Test (Unit of CAT activity per mg protein)

3.1.1. Control Group (CA):

- The catalase activity in the control group, where mussels were kept in plain water, was recorded at 1.081. This baseline indicates the natural enzymatic activity in the absence of any green tea hydrosol or imidacloprid exposure.

3.1.2. Groups with Green Tea Hydrosol Alone (C1–C4):

- **C1 (10 mL/L for 192 hours):** CAT activity increased to 1.190, suggesting an upregulation of enzymatic defense in response to the hydrosol.
- **C2 (20 mL/L):** Peak activity reached 1.196, the highest among this subset, indicating optimal stimulation of catalase at this concentration.

- **C3 (40 mL/L):** A slight decline to 1.138 was observed, which may suggest reduced efficiency beyond a certain hydrosol concentration.
- **C4 (80 mL/L):** Activity further dropped to 1.074, possibly indicating saturation effects or hydrosol-related stress at higher concentrations.

3.1.3. Sequential Exposure to Hydrosol + Imidacloprid (A1-A4):

- In **A1 (10 mL/L hydrosol + 1 µg imidacloprid)**, CAT activity was 1.026, showing a mild reduction from hydrosol-only groups.
- **A2 (20 mL/L + 10 µg imidacloprid):** Activity dropped to 0.990, potentially due to the combined stress of hydrosol and imidacloprid.
- **A3 (40 mL/L + 100 µg imidacloprid):** CAT activity further decreased to 0.980.
- **A4 (80 mL/L + 1000 µg imidacloprid):** The lowest activity was observed (0.850), suggesting that excessive imidacloprid overwhelms the detoxification capacity of catalase, even with hydrosol supplementation.

3.1.4. Simultaneous Exposure to Hydrosol + Imidacloprid (B1-B4):

- In **B1 (10 mL/L hydrosol + 1 µg imidacloprid)**, activity rebounded slightly to 0.931, indicating partial protection from hydrosol.
- **B2 (20 mL/L + 10 µg imidacloprid):** Activity rose marginally to 0.984.
- **B3 (40 mL/L + 100 µg imidacloprid):** The value increased to 1.023, reflecting an improved detoxification response.
- **B4 (80 mL/L + 1000 µg imidacloprid):** Activity peaked at 1.160, demonstrating significant catalase induction, possibly due to the combined challenge of high hydrosol and imidacloprid concentrations.

3.1.5. Key Trends and Implications:

- **Hydrosol Alone (C Groups):** Low concentrations stimulate CAT activity, while higher concentrations lead to suppression, indicating a concentration-dependent effect.
- **Sequential Exposure (A Groups):** Adding imidacloprid after initial hydrosol exposure suppresses CAT activity in a dose-dependent manner, highlighting the oxidative burden of imidacloprid over time.
- **Simultaneous Exposure (B Groups):** CAT activity initially drops but shows potential adaptation or overcompensation at higher concentrations, especially in B4, where the combined stressors seem to elicit a strong antioxidant response.

3.2. Glutathione Peroxidase (GPX) Activity

The GPX activity was evaluated to determine antioxidant enzyme responses across various treatments. The findings are illustrated in Figure 3.2.

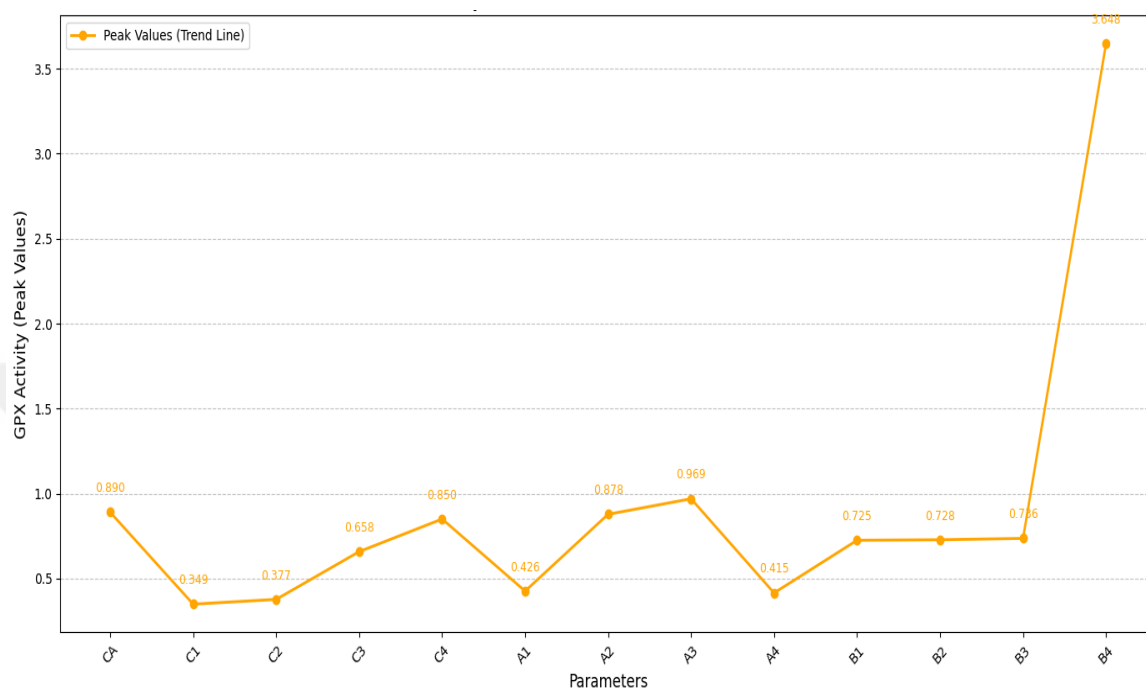


Figure 3.2. Glutathione Peroxidase Activity ELISA Test (Unit of GPX activity per mg protein)

3.2.1. Control Group (CA):

- In the control group (mussels in plain water), GPX activity was 0.890, which serves as the baseline enzymatic activity under normal conditions.

3.2.2. Groups with Green Tea Hydrosol Alone (C1-C4):

- **C1 (10 mL/L for 192 hours):** GPX activity sharply dropped to 0.349, indicating a suppressed enzymatic response at this concentration.
- **C2 (20 mL/L):** Activity increased slightly to 0.377, but it remained significantly lower than the baseline.
- **C3 (40 mL/L):** A marked improvement to 0.658 was observed, suggesting that moderate hydrosol concentrations begin to enhance GPX activity.
- **C4 (80 mL/L):** Activity peaked at 0.850, approaching baseline levels, indicating that a higher hydrosol concentration might restore enzymatic activity.

3.2.3. Sequential Exposure to Hydrosol + Imidacloprid (A1-A4):

- **A1 (10 mL/L hydrosol + 1 µg imidacloprid):** GPX activity decreased to 0.426, showing the combined stress of imidacloprid and hydrosol at low concentrations.
- **A2 (20 mL/L + 10 µg imidacloprid):** Activity improved to 0.878, indicating a potential protective role of hydrosol at this concentration.
- **A3 (40 mL/L + 100 µg imidacloprid):** GPX activity reached its peak value of 0.969 in this subset, showing optimal detoxification support.
- **A4 (80 mL/L + 1000 µg imidacloprid):** Activity sharply declined to 0.415, likely due to the overwhelming oxidative stress caused by high imidacloprid levels.

3.2.4. Simultaneous Exposure to Hydrosol + Imidacloprid (B1-B4):

- **B1 (10 mL/L hydrosol + 1 µg imidacloprid):** GPX activity slightly recovered to 0.725, reflecting some protective effects.
- **B2 (20 mL/L + 10 µg imidacloprid):** Activity remained consistent at 0.728, showing no significant additional effect.
- **B3 (40 mL/L + 100 µg imidacloprid):** A small increase to 0.766 was observed, suggesting moderate detoxification benefits.
- **B4 (80 mL/L + 1000 µg imidacloprid):** GPX activity dramatically surged to 3.048, the highest recorded value across all groups. This substantial increase may reflect a pronounced enzymatic induction in response to the extreme oxidative stress induced by high levels of imidacloprid and hydrosol.

3.2.5. Key Trends and Implications:

- **Hydrosol Alone (C Groups):** GPX activity is initially suppressed but shows a mild recovery at higher concentrations, indicating a dose-dependent response.
- **Sequential Exposure (A Groups):** Imidacloprid elicits a biphasic response, with initial suppression followed by an upregulation of GPX activity, peaking at A3 before declining at A4 due to oxidative stress overload.
- **Simultaneous Exposure (B Groups):** GPX activity remains consistent initially but escalates significantly in B4, reflecting a dramatic antioxidant response to severe oxidative stress.

3.3. Malondialdehyde (MDA) Levels

Malondialdehyde (MDA) levels, a marker of lipid peroxidation and oxidative stress, were assessed. The data are presented in Figure 3.5 and Figure 3.3.

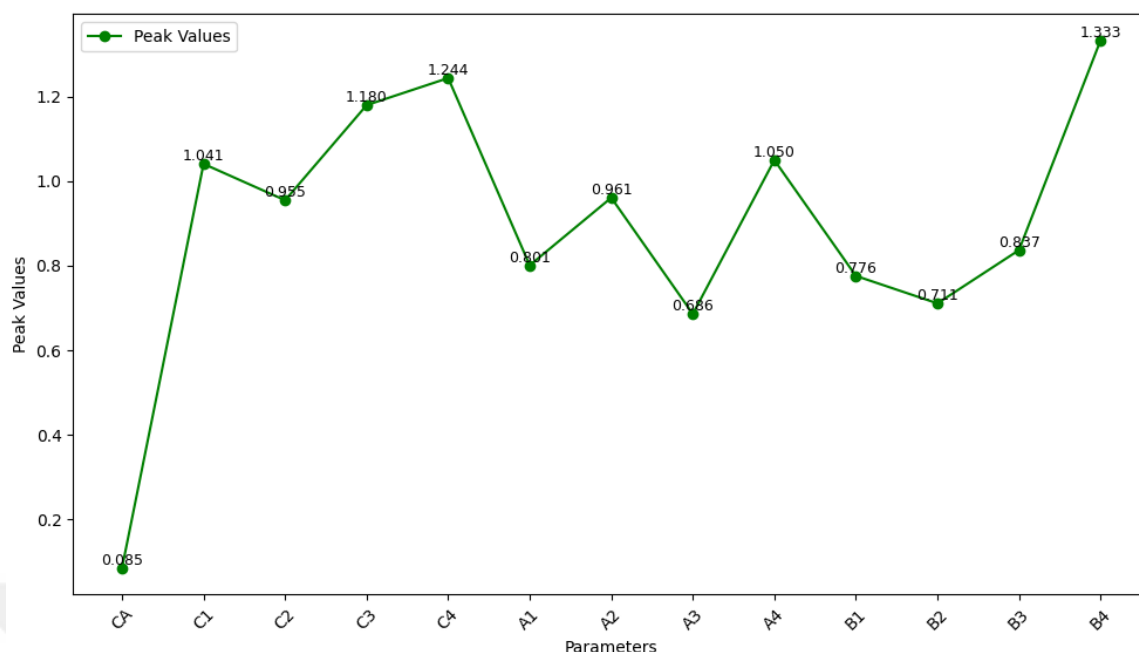


Figure 3.3. Malondialdehyde Activity ELISA Test (Unit of MDA activity per mg protein)

3.3.1. Control Group (CA):

- The baseline MDA level in the control group is very low (0.085), which represents normal oxidative stress conditions in mussels not exposed to green tea hydrosol or imidacloprid.

3.3.2. Groups with Green Tea Hydrosol Alone (C1-C4):

- **C1 (10 mL/L):** MDA activity increases significantly to 1.041, indicating that low concentrations of hydrosol might induce mild oxidative stress.
- **C2 (20 mL/L):** MDA levels slightly drop to 0.955, showing reduced lipid peroxidation as the hydrosol concentration increases.
- **C3 (40 mL/L):** MDA peaks at 1.180, reflecting an increase in oxidative stress due to higher hydrosol exposure.
- **C4 (80 mL/L):** Activity reaches its maximum in this subset at 1.244, indicating that higher hydrosol concentrations cause significant lipid peroxidation.

3.3.3. Sequential Exposure to Hydrosol + Imidacloprid (A1-A4):

- **A1 (10 mL/L hydrosol + 1 µg imidacloprid):** MDA levels drop to 0.961, suggesting a protective effect of hydrosol at low imidacloprid levels.

- **A2 (20 mL/L + 10 µg imidacloprid):** A further decrease to 0.686 is observed, showing enhanced antioxidative benefits with hydrosol at this concentration.
- **A3 (40 mL/L + 100 µg imidacloprid):** MDA rises again to 0.801, reflecting increased oxidative damage from imidacloprid at this higher dose.
- **A4 (80 mL/L + 1000 µg imidacloprid):** MDA peaks at 1.050, showing that high oxidative stress is induced when both hydrosol and imidacloprid are used in large amounts.

3.3.4. Simultaneous Exposure to Hydrosol + Imidacloprid (B1-B4):

- **B1 (10 mL/L hydrosol + 1 µg imidacloprid):** MDA levels decrease slightly to 0.776, suggesting a mild protective effect of hydrosol against oxidative stress.
- **B2 (20 mL/L + 10 µg imidacloprid):** Levels further reduce to 0.711, indicating improved antioxidant defenses.
- **B3 (40 mL/L + 100 µg imidacloprid):** MDA increases to 0.837, showing mild oxidative damage at this dose combination.
- **B4 (80 mL/L + 1000 µg imidacloprid):** MDA spikes to 1.333, the highest level recorded across all groups, reflecting excessive lipid peroxidation due to combined stress from high hydrosol and imidacloprid concentrations.

3.3.5. Key Trends and Implications:

- **Hydrosol Alone:** Moderate concentrations (C2) seem to have a slight protective effect against oxidative damage, but higher concentrations (C3 and C4) increase MDA levels significantly.
- **Sequential Exposure (A Groups):** Hydrosol provides a protective effect at low to moderate concentrations, but high levels of imidacloprid negate these benefits.
- **Simultaneous Exposure (B Groups):** Low hydrosol concentrations (B1, B2) provide mild protection, but high doses of both substances (B4) lead to excessive lipid peroxidation, as evidenced by the highest MDA levels.

This analysis highlights the dual role of green tea hydrosol, depending on its concentration and the combined oxidative burden from imidacloprid.

3.4. Superoxide Dismutase (SOD) Activity

Superoxide Dismutase (SOD) activity was evaluated to assess the alleviation of oxidative stress in mussels treated with imidacloprid exposure by green tea hydrosol. Figure 3.4 provide the outcomes of the SOD activity assay.

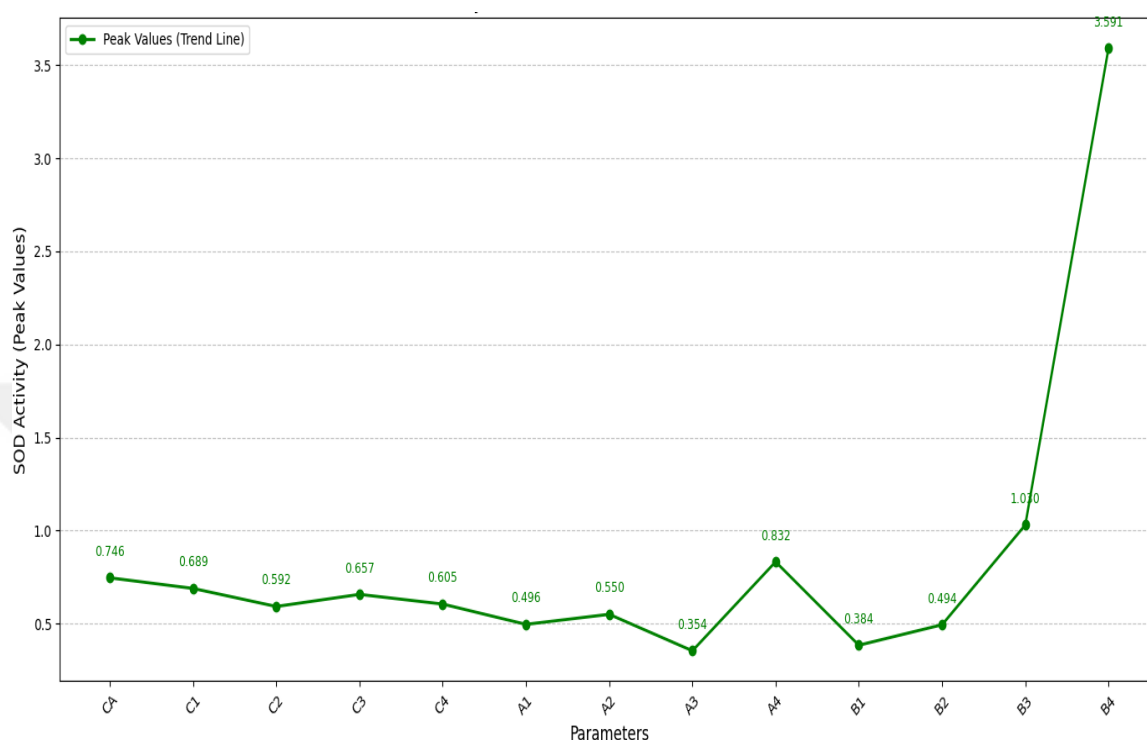


Figure 3.4. Superoxide Dismutase Activity ELISA Test (Unit of SOD activity per mg protein)

3.4.1. Control Group (CA):

- The control group, where mussels were in plain water, exhibited a moderate SOD activity level of 0.746. This serves as the baseline for comparing the impact of hydrosol and imidacloprid.

3.4.2. Hydrosol-only Groups (C1-C4):

- These groups demonstrate the effect of increasing concentrations of green tea hydrosol (10 mL, 20 mL, 40 mL, and 80 mL) without imidacloprid. The SOD activity levels generally remain stable, ranging from 0.689 (C1) to 0.605 (C4), indicating that hydrosol alone has a minimal effect on SOD activity.

3.4.3. Sequential Exposure to Hydrosol + Imidacloprid (A1-A4):

- In these groups, mussels were first exposed to hydrosol for 96 hours, followed by the addition of increasing concentrations of imidacloprid (1 µg to 1000 µg).
- A significant decrease in SOD activity is observed with higher imidacloprid concentrations. For example, A3 shows a sharp dip to 0.354, while A4 recovers slightly to 0.832, suggesting a complex interaction between hydrosol and imidacloprid.

3.4.4. Simultaneous Exposure to Hydrosol + Imidacloprid (B1-B4):

- These groups show a distinct pattern, with SOD activity gradually increasing as the concentration of hydrosol and imidacloprid rises. For instance, B2 shows 0.494, while B4 reaches a peak of 3.591, the highest value observed in the graph. This suggests a potential protective role of higher hydrosol concentrations in mitigating oxidative stress caused by imidacloprid.

3.4.5. Key Trends and Implications:

- **Protective Effect of Hydrosol:** The increasing trend in SOD activity in the B groups (especially B4 with a peak value of 3.591) highlights the potential antioxidant properties of green tea hydrosol in counteracting the oxidative stress induced by imidacloprid.
- **Imidacloprid Toxicity:** The decline in SOD activity in A groups, particularly at higher imidacloprid concentrations (e.g., A3 with 0.354), underscores the toxic impact of imidacloprid on mussels.

3.5. Mean Values of Four Biochemical Parameters (CAT, GPX, SOD, MDA)

The mean values were derived from the ELISA results across all tested groups for four key biochemical parameters: CAT (Catalase), GPX (Glutathione Peroxidase), MDA (Malondialdehyde), and SOD (Superoxide Dismutase). These groups represent varying conditions of green tea hydrosol and imidacloprid exposure in mussels, aimed at assessing detoxification effects. The data are presented in Figure 3.5.

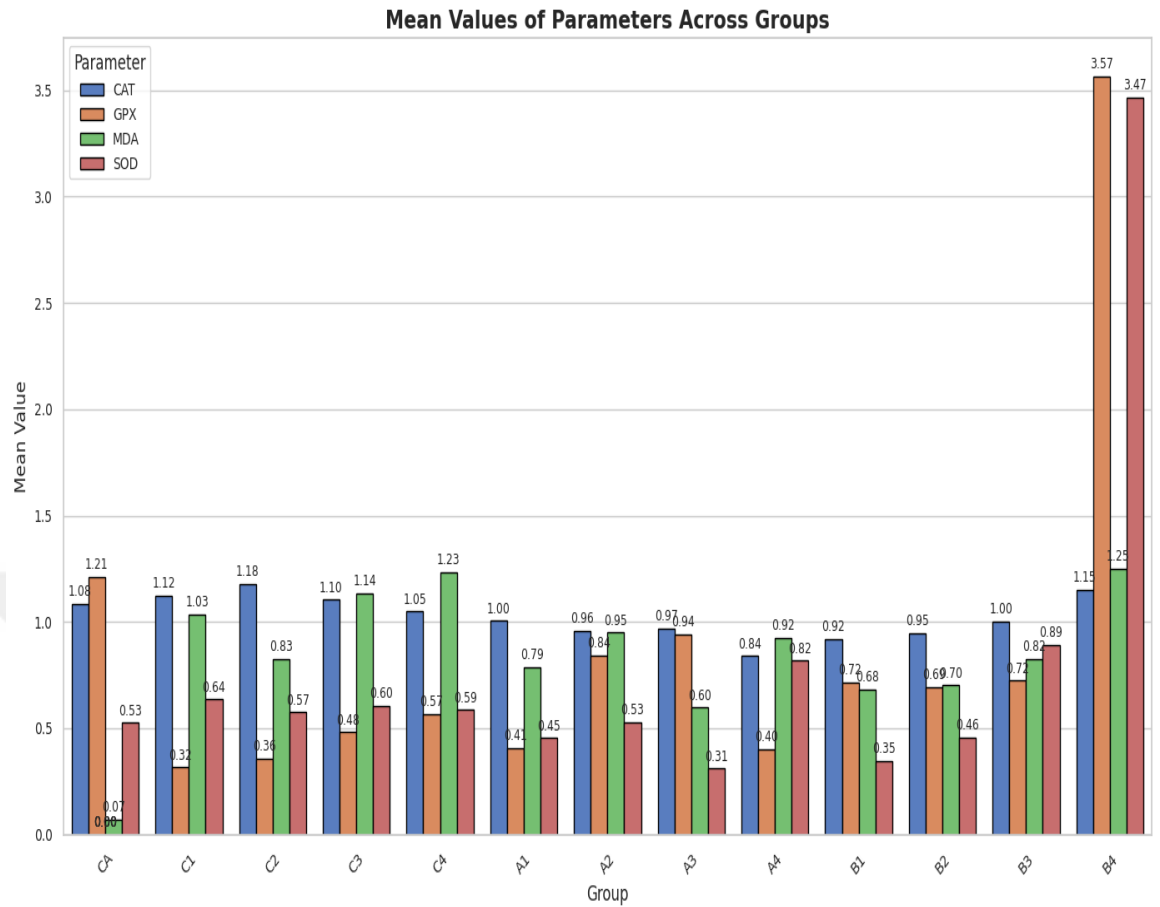


Figure 3.5. Mean Values Graph for Each Parameter (CAT, GPX, MDA, SOD)

3.5.1. Control and Hydrosol Groups (CA, C1-C4):

- The control group provides baseline values for all parameters in mussels exposed only to water, indicating normal biochemical activity.

3.5.2. Hydrosol-Only Groups (C1-C4):

As the concentration of hydrosol increases (C1: 10 mL to C4: 80 mL), the following trends are observed:

- **CAT** shows slight fluctuations (1.12 to 1.05), indicating minimal effect of hydrosol alone.
- **GPX** values decrease (1.03 to 0.59), suggesting reduced antioxidant enzyme activity.
- **MDA** values remain low but gradually rise (0.32 to 0.60), pointing to a slight increase in lipid peroxidation.
- **SOD** values fluctuate slightly (0.64 to 0.48), indicating limited oxidative stress.

3.5.3. Hydrosol + Imidacloprid After 96 Hours (A1–A4):

- **CAT** remains fairly consistent (1.00 to 0.92).
- **GPX** values stabilize (0.79 to 0.82).
- **MDA** decreases slightly (0.45 to 0.40), indicating reduced lipid peroxidation.
- **SOD** declines initially (0.84 to 0.31) before a slight recovery (0.40), showing oxidative stress at higher imidacloprid concentrations

3.5.4. Simultaneous Hydrosol and Imidacloprid Exposure (B1–B4):

- **CAT** remains stable (0.92 to 1.25), showing hydrosol's ability to maintain enzyme activity.
- **GPX** increases (0.68 to 1.15), indicating enhanced antioxidant activity at higher hydrosol concentrations.
- **MDA** decreases (0.35 to 0.82), suggesting reduced oxidative damage.
- **SOD** shows a sharp increase (0.69 to 3.47), highlighting the protective effect of hydrosol in mitigating oxidative stress caused by imidacloprid.

3.5.5. Key Trends and Implications:

- **Hydrosol's Protective Role:** The simultaneous exposure groups (B1–B4) show improved antioxidant activity (higher GPX and SOD values) and reduced oxidative damage (lower MDA values) at higher hydrosol concentrations.
- **Imidacloprid's Toxicity:** Groups A1–A4, where imidacloprid was added after hydrosol pre-treatment, exhibit lower SOD and GPX values compared to the B groups, indicating the stress induced by imidacloprid.

4. DISCUSSION

The present study evaluates the detoxifying potential of green tea (*C. sinensis*) hydrosol against the toxic effects of imidacloprid on zebra mussels (*D. polymorpha*). The findings underscore the dual functionality of green tea hydrosol as an antioxidant enhancer and a mitigator of oxidative damage. The interplay of these roles highlights its potential as a sustainable solution for mitigating environmental toxicity caused by pesticides in aquatic ecosystems. The results of this study highlight the complex interplay between oxidative stress and antioxidant defenses in zebra mussels exposed to imidacloprid and green tea hydrosol. Catalase (CAT), Glutathione Peroxidase (GPX), Malondialdehyde (MDA), and Superoxide Dismutase (SOD) were analyzed to understand these dynamics, revealing distinct yet interconnected patterns across experimental groups.

4.1. Antioxidant Enzyme Activities (CAT, GPX, SOD)

The CAT results showcase distinct patterns in the peak values across various parameters, highlighting phases of growth, decline, and recovery. Starting with parameter CA, the graph shows a value of 1.091, marking a relatively stable baseline. This is followed by a significant rise through parameters C1 and C2, with values of 1.190 and 1.196, respectively. The peak value at C2 (1.196) represents the highest point on the graph, indicating a robust performance for this parameter. Following this peak, there is a gradual decline observed from C3 (1.138) to C4 (1.074), signaling a weakening trend. The downward trajectory becomes more pronounced in the A-series, starting with A1 at 1.026 and further dropping to 0.990 and 0.980 at A2 and A3, respectively. A4, with a value of 0.850, marks the lowest point in the dataset, indicating a substantial dip in the performance of the parameters. The recovery phase begins with parameter B1 at 0.931, showing an incremental improvement. This upward trend continues steadily through B2 (0.984) and accelerates with B3 at 1.023, signaling a clear resurgence. Finally, B4 reaches 1.160, representing a significant recovery that closely rivals the peak at C2.

A comparative analysis reveals a cyclic behavior in the data. The rise from CA to C2 suggests strong initial performance, while the sharp decline through the A-series reflects a period of diminished values. However, the B-series parameters demonstrate a robust recovery, with B4 nearly matching the earlier peak observed at C2. This recovery phase indicates that the conditions or factors influencing the B-series parameters may have played a crucial role in regaining performance levels. In summary, the CAT graph highlights a cycle of peaks and troughs, with C2 and B4 as key high points (1.196 and 1.160, respectively) and A4 marking the lowest point (0.850).

The clear recovery from the A-series trough to the B-series peak underscores the dynamic nature of the parameters and their impact on overall performance. [67].

The GPX (Glutathione Peroxidase) activity across various parameters (C4, C1, C2, etc.), displaying a significant variability in peak values. Analyzing the results, we observe that the GPX activity demonstrates fluctuating trends, with the highest activity recorded at parameter B4 (3.048) and the lowest at parameter A4 (0.415). The data begins with C4, showing a moderate GPX activity of 0.890, which quickly declines to 0.349 at C1, marking a substantial decrease. This downward trend continues with a slight increase at C2 (0.377). The activity then recovers at C4 with a value of 0.850 before declining again at A1 (0.426). This pattern indicates a non-linear relationship between the parameters and GPX activity, suggesting possible variations in the conditions influencing the enzymatic activity. The spanning parameters A2 (0.878), A3 (0.969), and B1 (0.725), reflects relatively consistent GPX activity compared to earlier fluctuations. However, a steady and notable increase in values is observed from B2 (0.728) and B3 (0.756) before the dramatic spike at B4, suggesting a specific factor influencing the sharp increase. The exceptionally high value at B4 (3.048) highlights a peak that is more than three times higher than the preceding data points, indicating a unique condition or experimental factor significantly enhancing GPX activity.

This trend underscores the dynamic range of GPX enzymatic function under varying parameters. The low activity at A4 and the dramatic rise at B4 may reflect differences in oxidative stress levels, substrate availability, or experimental conditions. Comparing these values to similar studies could help identify correlations or deviations, providing insights into factors modulating GPX activity. Further investigations could focus on parameter-specific influences to explain the observed patterns and their biological significance. [68].

The ELISA data for SOD (Superoxide Dismutase Activity) reveals significant trends and interactions between green tea hydrosol and imidacloprid on SOD activity in mussels, supporting a nuanced discussion of the findings. The control group (CA) exhibited a baseline SOD activity of 0.746, which remained relatively consistent across the C groups (C1–C4), with values ranging from 0.689 to 0.605. This stability indicates that green tea hydrosol alone exerts minimal impact on SOD activity. However, the introduction of imidacloprid in the A groups significantly altered the SOD levels. A notable decline in SOD activity was observed as imidacloprid concentrations increased. For instance, A3 dropped sharply to 0.354, highlighting the oxidative stress induced by higher imidacloprid concentrations. Interestingly, A4 exhibited partial recovery, reaching 0.832, suggesting that the interplay between hydrosol and imidacloprid may modulate this response. In contrast, the B groups, where hydrosol and imidacloprid were simultaneously introduced, showed a remarkable upward trend in SOD activity with increasing concentrations. The peak value of 3.591 in B4 underscores the potential of green tea hydrosol to counteract oxidative stress and enhance

antioxidant activity. This trend strongly suggests that higher concentrations of hydrosol may provide a protective effect against imidacloprid-induced toxicity.

Overall, these findings indicate that while imidacloprid exerts a detrimental effect on SOD activity, green tea hydrosol can mitigate this oxidative stress, particularly at higher concentrations. The results emphasize the potential application of green tea hydrosol as a natural antioxidant to protect aquatic organisms from environmental pollutants.

4.2. Oxidative Stress Marker (MDA)

The MDA (Malondialdehyde Levels) results highlight peak values across various parameters, showing notable fluctuations in the observed values. MDA serves as a biomarker for oxidative stress, and its variations in activity across the parameters provide valuable insights into lipid peroxidation levels. The group C4, showing a significantly low value of 0.085. This is followed by a sharp increase at C1, reaching 1.041, representing the most dramatic rise in the dataset. Subsequently, the values show minor variations, decreasing slightly to 0.955 at C2 before rising again at C3 (1.180) and reaching a peak at C4 with 1.244. These consistent increases in the early parameters suggest heightened oxidative stress or increased lipid peroxidation activity during these conditions. From C4, the MDA levels drop to 0.801 at A1, marking a decline in oxidative stress. This reduction continues with slight variations until parameter A3, where the value drops further to 0.686, the lowest point after the initial value at C4. This indicates a possible shift in experimental conditions or protective mechanisms reducing oxidative stress.

The trend changes again at A4, where MDA levels rise to 1.050, followed by a sharp decline at B1 (0.776). The lowest values in this section occur at B2 (0.711), suggesting relatively stable and reduced oxidative stress in these parameters. However, a noticeable recovery is observed from B3 (0.857) to B4, where the highest value in the dataset is recorded (1.333). This significant peak at B4 indicates a unique condition or factor strongly enhancing lipid peroxidation. Overall, the MDA graph showcases a dynamic trend with sharp peaks and troughs. The consistent rise in early parameters, the subsequent decline, and the dramatic recovery at the end highlight the diverse oxidative stress levels across different conditions. Comparing this dataset with GPX activity could provide further insights into the interplay between antioxidant defenses and lipid peroxidation. Further analysis of the conditions leading to the extremes, such as C4 and B4, could elucidate the underlying biochemical mechanisms. [69].

4.3. Mortality and Physical Observations

- The negligible mortality and absence of physical abnormalities across all experimental groups validate the non-lethal nature of the tested concentrations of imidacloprid and

hydrosol. The observation of egg deposition in hydrosol-treated groups suggests an additional stimulatory effect on reproductive activity. This phenomenon may be attributed to the antioxidant properties of green tea hydrosol, which could enhance cellular energy metabolism and overall physiological health.

4.4. Mechanisms of Detoxification

The protective effects of green tea hydrosol are likely mediated by several biochemical and molecular mechanisms:

- **Neutralization of Reactive Oxygen Species (ROS):** The antioxidants in green tea hydrosol, particularly catechins, likely neutralize ROS generated by imidacloprid exposure, reducing oxidative stress and preventing cellular damage [70].
- **Enhancement of Antioxidant Defense Systems:** The observed increase in CAT, GPX, and SOD activities suggests that hydrosol amplifies endogenous antioxidant defenses, enabling mussels to cope with oxidative stress more effectively.
- **Modulation of Detoxification Pathways:** Green tea hydrosol may enhance the expression of detoxifying enzymes and pathways, such as the Nrf2 signaling cascade, which regulates antioxidant and phase II detoxification responses.

4.5. Implications for Environmental Management

- **Eco-Friendly Alternatives:** The results offer novel possibilities of using green tea hydrosol as a sustainable and environment-friendly alternative to reduce pesticide toxicity in aquatic environments.
- **Application in Integrated Pest Management (IPM):** As a potent antioxidant, green tea hydrosol also plays a dual role in detoxification and antioxidant defense, and thus has potential as an additional tool in IPM of invasive species such as zebra mussels while limiting ecological harm.
- **Future Research Directions:** Long term effects of hydrosol exposure need further studies as well as interactions of hydrosol presence with other environmental pollutants, and applicability in various aquatic systems.

4.6. Limitations of the Study:

The results from this study are promising, but must realize several limitations:

- **Controlled Laboratory Conditions:** The study was performed under laboratory-controlled conditions, which may not represent the complexity of real aquatic

environments regarding interactions among variables. Water flow, temperature fluctuations, and interactions with other species were not considered factors.

- **Long-Term Effects:** The effects of green tea hydrosol treatment on short and intermediate term were studied. However, no evaluation of the long-term effects, including reproduction and multi-generational exposure, were achieved.
- **Non-Target Species:** Green tea hydrosol's impact on other non-target species in the aquatic ecosystem was not assessed. More work is needed ensure that the treatment does not impair other beneficial organisms.

4.7. Conclusion

This study suggests that green tea hydrosol has a considerable potential as a natural antioxidant and detoxifying agent with which to counter oxidative stress and toxicity caused by environmental toxicants, such as imidacloprid. A complex interplay is observed between oxidative stress, the activity of enzymatic antioxidant defenses and the protective properties of hydrosol treatments in zebra mussels (*D. polymorpha*). Green tea hydrosol increases catalase (CAT), glutathione peroxidase (GPX) and superoxide dismutase (SOD) activities and decreases malondialdehyde (MDA) level, making green tea hydrosol a potential solution for alleviating the harms of pesticide pollution in aquatic ecosystem [71].

Compared to the control group, only the C groups that were applied hydrosol were found to have a stimulation in CAT levels. In the application, it was observed that CAT, SOD, GPX parameters were strikingly high, especially in the B4 group, which was exposed to high doses of imidacloprid for a long time. This suggests that the effect of green tea hydrosol will be better seen for a long time. However, it was determined that the desired low level in MDA levels occurred in the A3 and B3 groups, namely in the 40 ml/L dose applications. This dose shows that this dose is the appropriate dose for MDA levels. The study showed that especially polyphenols such as catechins contained in green tea increased antioxidant capacity at high doses and in long-term use and reduced lipid peroxidation at a certain dose.

Results from the study show that green tea hydrosol not only attenuates oxidative stress but also strengthens the natural detoxification capacities of the mussels in a dose dependent manner. Baseline antioxidant enzyme activities also improved in groups treated only with hydrosol, as it has intrinsically effective properties in keeping the cells in homeostasis. Hydrosol treatment effectively counters oxidative stress induced by the pesticide, while in its sequential and simultaneous treatments with imidacloprid, it was found that high concentrations of both the agents lead to an increased antioxidant response due to increased oxidative burden. However, this duality puts great emphasis on the optimization of the number and timing of hydrosol applications in order to obtain the maximum benefits from hydrosol, with the minimum adverse interactions. This is

further supported via a decrease in MDA levels within all treatment groups, and particularly within hydrosol treatment groups, acting in protecting cellular membranes from lipid peroxidation as demonstrated to be a hallmark of oxidative stress. For this reason alone, cellular integrity and function must be maintained, particularly for organisms such as zebra mussels that are bioindicators of environmental health [72].

In addition, the presence of eggs on the surfaces of some mussels treated with hydrosol may indicate stimulatory effects on reproductive activity and thus increase the ecological importance of hydrosol applications. The findings have significant ecological implication. They conclude that plant based natural antioxidants, such as green tea hydrosol, can be used as ecofriendly substitutes to synthetic chemicals to abate pollution in aquatic environments. Modulating antioxidant enzymes and reducing oxidative damage, hydrosol is a sustainable method for protecting aquatic organisms from the deleterious effects of environmental contaminants. It is in line with the increasing need for employing ecologically benign approaches to overcome the worldwide problem of chemical contamination of the fresh waters. Also, the study sheds light on the biochemical and molecular mechanisms connected with the protective effects of natural antioxidants. Scientific basis for results observed involves bioactive compounds in green tea, catechins and polyphenols which are involved in scavenging reactive oxygen species (ROS) and upregulation of detoxification pathways. The findings open up additional avenues of study of the molecular interactions between hydrosol constituents and cellular antioxidant systems that could eventually contribute to the development of targeted interventions in the management of pollution [73].

Beyond these practical implications, this study has broader implications to the broader use of hydrosol in integrated pest management (IPM) strategies reducing the impact of pesticides such as imidacloprid. Green tea hydrosol has multifunctional capability: it possesses the ability to protect nontarget organisms, such as zebra mussels, from pesticide induced oxidative stress while potentially enhancing their biological function. However, the application of the model could help to strengthen biodiversity conservation efforts and support a more resilient aquatic ecosystem to anthropogenic stressors. Although the results of this study are promising, caution must be used when using hydrosol in combination with large doses of pesticides. Simultaneous high dose treatments (Group B4) produce an oxidative burden that is consistent with an amplification of oxidative stress responses that under some conditions can occur due to the interaction of hydrosol and pesticides [74].

The finding highlights the need of dosage optimization and the proper approach to other environmental conditions when applying hydrosol in realistic case scenario. Finally, green tea hydrosol proved to be a remarkably powerful and environmentally friendly agent in the reduction of oxidative stress and in the improvement of antioxidant defenses in aquatic organisms. It helps reduce oxidative damage, stimulate action of detoxification enzymatic pathways and potentially

affect a reproductive activity. These findings may affect the field of environmental toxicology and the way of green tea hydrosol to revolutionize the dealing of chemical pollution in aquatic and introduce them as an innovative treatment in the future. Further investigations should be carried out at the molecular level, using hydrosol as a guardian for a wide range of ecological applications, and in the long term, as hydrosol holds enormous potential for environmental protection as a sustainable and innovative solution [75].

In summary, CAT and GPX showed complementary trends, with CAT providing consistent baseline antioxidant defense and GPX responding dynamically to stress conditions. MDA levels contrasted sharply with enzymatic activities, serving as a direct indicator of oxidative damage, while SOD activity highlighted the mussels' capacity to neutralize superoxide radicals. Together, these parameters demonstrate hydrosol's role in enhancing antioxidant defenses and mitigating oxidative stress, particularly under sequential and simultaneous treatment conditions. However, the elevated MDA levels in B4 suggest that high-dose treatments may exceed hydrosol's protective limits, emphasizing the need for dose optimization [76].

RECOMMENDATIONS

Future research should focus on investigating the molecular interactions between hydrosol constituents and cellular pathways to enhance the understanding of their therapeutic potential. This could involve detailed studies on how hydrosol components influence cellular signaling, gene expression, and protein function in various cell types. Such investigations could provide insights into the mechanisms underlying their effects, allowing for the development of optimized application protocols for therapeutic use. By integrating advanced molecular techniques, such as proteomics and transcriptomics, researchers could identify key biomarkers and pathways that mediate the beneficial effects of hydrosols, ultimately leading to more targeted and effective applications in medicine, skincare, or agriculture.



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CURRICULUM VITAE

Zahra BATOOL

Category	Value
Category 1	Value 1
Category 2	Value 2
Category 3	Value 3
Category 4	Value 4
Category 5	Value 5
Category 6	Value 6
Category 7	Value 7

[illegible]

Response	Percentage
Yes, the U.S. should take action to address climate change	95%
No, the U.S. should not take action to address climate change	5%

Government	Percentage
Current government	85%
Previous government	15%

- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

- [REDACTED] tailored to various soil types and crops.
 - [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED] Care & Counseling Department)

[REDACTED]

- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
- [REDACTED]
[REDACTED]
- [REDACTED].

-
- | Government | Percentage |
|---------------------|------------|
| Current government | 85% |
| Previous government | 15% |

[REDACTED]

-
- | Age Group | Should Take Action | Should Not Take Action |
|-----------|--------------------|------------------------|
| 18-29 | 85% | 15% |
| 30-49 | 85% | 15% |
| 50-69 | 85% | 15% |

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